

Original article

An experimental and modeling-based approach to locate IgE epitopes of plant profilin allergens

Gema López-Torrejón, PhD,^a Araceli Díaz-Perales, PhD,^a Julia Rodríguez, MD, PhD,^b
Rosa Sánchez-Monge, PhD,^a Jesus F. Crespo, MD, PhD,^b Gabriel Salcedo, PhD,^a
and Luis F. Pacios, PhD^c Madrid, Spain

Background: Plant profilins are actin-binding proteins that form a well-known panallergen family responsible for cross-sensitization between plant foods and pollens. Melon profilin, Cuc m 2, is the major allergen of this fruit.

Objective: We sought to map IgE epitopes on the 3-dimensional structure of Cuc m 2.

Methods: IgE binding to synthetic peptides spanning the full Cuc m 2 amino acid sequence was assayed by using a serum pool and individual sera from 10 patients with melon allergy with significant specific IgE levels to this allergen. Three-dimensional modeling and potential epitope location were based on analysis of both solvent exposure and electrostatic properties of the Cuc m 2 surface.

Results: Residues included in synthetic peptides that exerted the strongest IgE-binding capacity defined 2 major epitopes (E1, consisting of residues 66-75 and 81-93, and E2, consisting of residues 95-99 and 122-131) that partially overlapped with the actin-binding site of Cuc m 2. Two additional epitopes (E3, including residues 2-10, and E4, including residues 35-45) that should show weaker putative antigen-antibody associations and shared most residues with synthetic peptides with low IgE-binding capacity were predicted on theoretical grounds.

Conclusions: Strong and weak IgE epitopes have been uncovered in melon profilin, Cuc m 2.

Clinical implications: The different types of IgE epitopes located in the 3-dimensional structure of melon profilin can constitute the molecular basis to explain the sensitization and cross-reactivity exhibited by this panallergen family. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Profilin, melon allergy, cross-reactivity, 3-dimensional modeling, solvent exposure, electrostatic potential, allergen surface, IgE epitope

Abbreviations used

3-D: Three-dimensional
PB: Poisson-Boltzmann
PDB: Protein Data Bank
PLP: Poly-L-proline
SES: Solvent-excluded surface

Plant profilins represent an extensively studied pan-allergen family present in most plant allergenic sources thus far investigated, including plant foods, pollens, and latex.^{1,2} Profilin allergens are highly conserved (>70% of amino acid sequence identity in most pair comparisons) and therefore induce cross-sensitization between plant allergenic sources. However, they are usually recognized by IgE of only 10% to 30% of patients with allergy to plant food and pollen.² In contrast, melon (*Cucumis melo*) profilin, Cuc m 2, evoked positive *in vivo* (skin prick test) responses in more than 75% of the patients allergic to this fruit,³ thus being a major and the most relevant melon allergen described at present. Interestingly, allergy to melon is commonly associated with oral allergy syndrome and multiple pollen reactivities, both characteristics linked to profilin allergens.^{2,4}

Profilins are around 13- to 14-kD proteins (131-134 amino acid residues) involved in the regulation of the actin cytoskeleton.⁵⁻⁷ Besides actin, profilins bind poly-L-proline (PLP) and phosphatidyl inositol-4,5-bisphosphate, a component of cell-signaling transduction pathways. Binding sites for these ligands have been identified in the 3-dimensional (3-D) structure of plant profilins, together with a plant-specific binding pocket located near the actin-binding surface, which is not present in profilins from other organisms.^{8,9} The availability of crystal structures of plant profilins from *Arabidopsis thaliana* pollen (Ara t 8),⁸ birch pollen (Bet v 2),⁹ and *Hevea brasiliensis* latex (Hev b 8.0204; Protein Data Bank [PDB] code 1G5U) has triggered research activity to identify antigenic sites. Besides these experimental structures, other homology-modeled theoretical structures from a variety of plant sources¹⁰⁻¹³ have been studied to determine IgE-binding epitopes. The large extent of cross-reactivity among plant profilins justifies the use of a single profilin for diagnosis:

From ^aUnidad de Bioquímica, Departamento de Biotecnología, E. T. S. Ingenieros Agrónomos; ^bServicio de Alergia, Hospital Universitario Doce de Octubre; and ^cUnidad de Química y Bioquímica, Departamento de Biotecnología, E. T. S. Ingenieros de Montes.

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Reprint requests: Luis F. Pacios, PhD, Unidad de Química y Bioquímica, E.T.S. Ingenieros de Montes, Ciudad Universitaria, 28040 Madrid, Spain.
E-mail: luis.fpacios@upm.es.

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recombinant birch pollen profilin, Bet v 2, is currently widely used as the allergen of choice.^{13,14} However, many reports have also shown only partial cross-reactivity between plant profilins,^{12,13} whereas other studies have revealed the existence of fine specificity of IgE to variable epitopes of plant species, even within the same family.¹⁵

There is considerable evidence in favor of conformational instead of linear IgE epitopes in profilins allergens.⁸⁻¹³ Potential epitopes on plant profilins have been predicted to overlap with ligand (actin and PLP)-binding sites^{8,9,11} or to be located near the plant-specific binding pocket.⁹ However, with few exceptions,¹³ the physical properties of the surface that might form an antigenic site have not been addressed. It is frequent to find in the literature mere schematic cartoon representations of possible epitopes without considering essential properties to study the potential antigenic behavior of a surface region as the electrostatic potential and solvent exposure.¹⁶⁻¹⁸ The study of these properties on the structures of plant profilins can help to uncover both specific and cross-reactive IgE epitopes. These theoretical predictions can then be tested by using an experimental and complementary method as the analysis of IgE-binding properties of synthetic peptides covering the amino acid sequence of the allergen.

Here we report the identification of potential epitopes of melon profilin, Cuc m 2, which was selected as a profilin allergen model, by using a dual and complementary approach based on its 3-D modeling and synthetic peptide analysis.

METHODS

Patients and sera

Sera from 10 patients with allergy to melon selected at the Food Allergy Unit of the Hospital Universitario Doce de Octubre (Madrid) were used. All patients showed a convincing clinical history of allergic reactions after melon ingestion and a positive prick-by-prick response to fresh melon. The presence of melon allergy was further ascertained on the basis of a positive result on double-blind, placebo-controlled food challenge (37.5-200 g of melon), which was carried out as previously reported.⁴ All 10 patients presented with oral allergy syndrome, with additional symptoms in some cases (epigastralgia in 2 patients and bronchial spasm, exanthema, macroglossia, and angioedema in 1 patient each). All selected patients showed also multiple reactivities to pollens, mainly from grasses and olive tree. A pool of sera from 5 atopic subjects with allergy to house dust mites but not to plant foods or pollens was used as a negative control. Written informed consent was obtained from both patients and control subjects.

Heterologous expression and isolation of recombinant Cuc m 2

Expression of melon profilin, Cuc m 2, in *Escherichia coli* XL1-Blue cells and isolation of the recombinant allergen were performed by using methods previously reported.³ Natural Cuc m 2 was purified as in López-Torrejón et al.³ Isolated allergens were quantified by means of the commercial bicinchoninic acid test (Pierce, Cheshire, United Kingdom).

Specific IgE determination

Specific IgE binding to natural and recombinant Cuc m 2 was carried out as previously described¹⁹ by using 10 individual sera from

patients with melon allergy (1:3 dilution) and both purified allergen forms as solid phase (2 µg/mL). BSA (1% in PBS buffer; mean [n = 7] OD = 0.081 units), and a serum pool from control subjects (OD <0.05 units when tested toward natural or recombinant Cuc m 2) were tested as negative controls. All tests were performed in triplicate.

Immunodetection assays

Samples (3 µg of natural Cuc m 2 and recombinant Cuc m 2 and 3 µg of ovalbumin [Sigma-Aldrich, Steinheim, Germany]) were separated by means of SDS-PAGE on 15% polyacrylamide gels under nonreducing conditions and then electrotransferred onto polyvinylidene difluoride membranes. Membranes were then incubated with a serum pool from patients allergic to melon (1:3 dilution); treated with mouse anti-human IgE mAb HE-2 ascitic fluid (1:3000 dilution),²⁰ a rabbit anti-mouse IgG peroxidase-conjugated antibody (Dako A/S, Denmark; 1:5000 dilution); and finally revealed by means of enhanced chemiluminescence according to the manufacturer's instructions (ECL-Amersham Biosciences, Little Chalfont, United Kingdom).

Dot-blot analysis of solid phase-bound synthetic peptides

Twenty-six synthetic decapeptides overlapping by 5 amino acids spanning the complete Cuc m 2 protein sequence and covalently bound to a cellulose membrane (SPOTs) were obtained from JPT Peptide Technologies (Berlin, Germany). Five different SPOT membranes were blocked with PBS buffer containing 5% BSA for 3 hours at 25°C and then incubated overnight with the serum pool or 4 individual sera (1:3 dilution) from patients with melon allergy, respectively. After washing, a mouse anti-human IgE mAb HE-2 ascitic fluid,²⁰ a rabbit anti-mouse IgG-peroxidase-conjugated antibody, and the ECL Amersham chemiluminescence kit to reveal IgE-binding peptides were sequentially added, as described above.

Homology modeling

The 3-D structure of Cuc m 2 was modeled by using the Swiss-Model Protein Modelling Server,²¹ taking as templates the following crystal structures of plant profilins: (1) *A thaliana* pollen, Ara t 8, PDB code 1A0K⁸ (76.2% of sequence identity); (2) birch pollen, Bet v 2, PDB code 1CQA⁹ (78.9% identity); and (3) *H brasiliensis*, Hev b 8.0204, PDB code 1G5U (83.1% identity). Secondary structure was identified with the DSSP program.²²

Protein surfaces and electrostatic potentials

Solvent-excluded surfaces (SESs) were obtained with PyMOL 0.99 (pymol.sourceforge.net). SES areas and relative accessibility values of each residue with respect to reference data were computed with Arvomol 4.0,²³ as explained previously,²⁴ to obtain the profile of percentage of solvent exposure. The term "molecular surface" is used as synonymous with SES.

Electrostatic Poisson-Boltzmann (PB) potentials were obtained with the APBS 0.4.0 software,²⁵ assigning AMBER99²⁶ charges and radii to all the atoms, including hydrogens, which were added and optimized with PDB2PQR.²⁷ Fine grid spacings of 0.35 Å around the 1957 atoms were used to solve the linearized PB equation in sequential-focusing multigrid calculations in a mesh of 161 points per dimension at 298.15 K. Dielectric constants were 2 for the protein and 78.54 for water. The output mesh was processed in scalar OpenDX format to render isocontours and mappings onto the surfaces with PyMOL 0.99. Potential values are given in units of kT per unit charge (k, Boltzmann's constant; T, temperature).

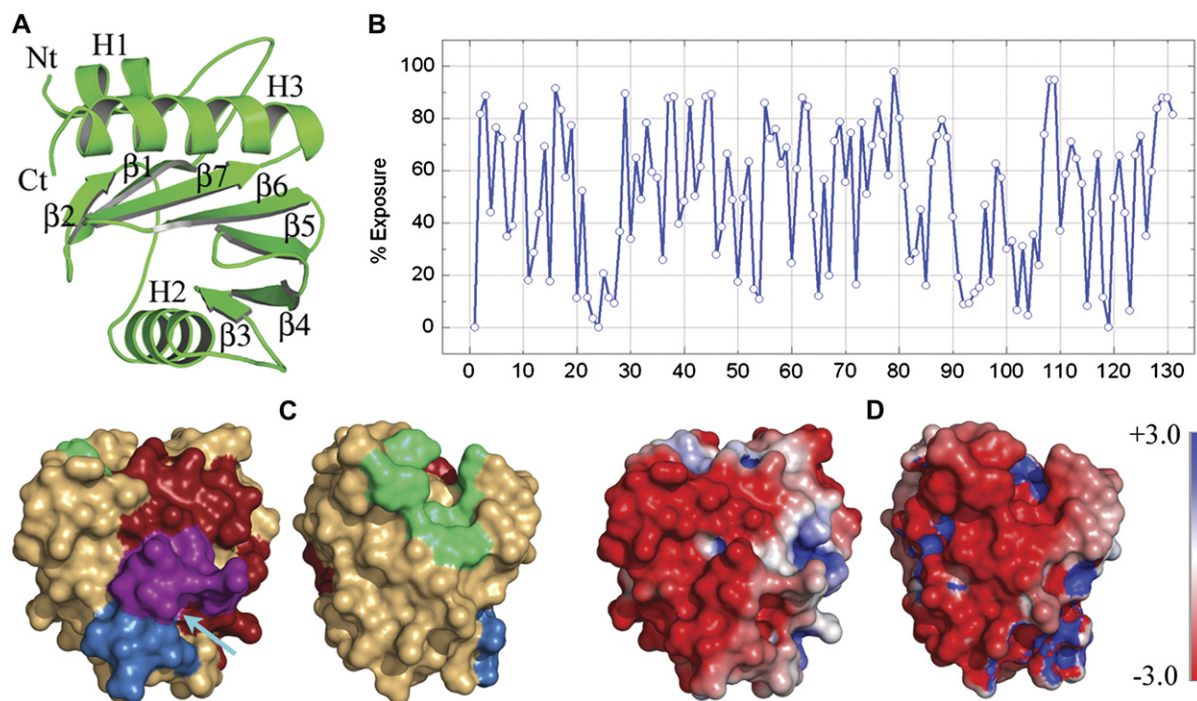


FIG 1. **A**, Ribbon diagram of Cuc m 2. **B**, Profile of percentage of solvent exposure. **C**, Surface at the orientation in Fig 1, **A** (left), and after a counterclockwise 120° rotation about a vertical axis (right) showing (1) PLP-binding (green) and (2) actin-binding (red) sites and (3) plant-specific pocket (blue). Overlap between 2 and 3 is colored purple. **D**, PB potential mapped onto surfaces.

RESULTS

Structure, surface, and electrostatic potential of Cuc m 2

The secondary structure of the modeled geometry of Cuc m 2 (Fig 1, A) showed the profilin motif^{8,9} formed by 7 β -strands sandwiched between the N- and C-terminal, nearly parallel α -helices H1 (residues 3-12) and H3 (112-128) on one side and the middle perpendicular helix H2 (44-55) on the other side. The strands were composed of the following residues: β 1, 22 to 27; β 2, 32 to 35; β 3, 65 to 67; β 4, 70 to 74, β 5, 82 to 87; β 6, 90 to 96; and β 7, 100 to 106. The high sequence identity between Cuc m 2 and the template profilins implies a great similarity with these crystal structures, and hence the model structure of Cuc m 2 can be considered accurate enough for ligand-binding sites and epitope identification.

We identified ligand (actin and PLP)-binding sites characteristic of profilins by comparing the profile of exposure to the solvent (Fig 1, B) and the molecular surface (Fig 1, C) of Cuc m 2 with those of the template profilins (not shown). The major actin-binding site consisted of strands β 4, β 5, and β 6; loops β 4 β 5 and β 5 β 6; and the half of helix H3 nearest to these loops.^{8,9,28} After considering possible individual amino acid contributions to the exposed surface, this site was identified as the connected surface (red and purple in Fig 1, C) composed of the following residues: 71, 73 to 82, 84, 86 to 89, 95, 111 to 114, 116, 117, 120, and 121. The PLP-binding site

spanned an accessible hydrophobic patch (green in Fig 1, C) near the outer ends of the N- and C-terminal helices, was located at the opposite side of the actin-binding site, and consisted of the following residues: W3 (88% exposure), Y6 (72% exposure), I25 (21% exposure), W33 (78% exposure), Y125 (73% exposure), and L126 (35% exposure). The third site is the plant-specific binding pocket identified in *Arabidopsis* profilin⁹ as the region composed of residues 53 to 58 (blue in Fig 1, C) and 75 to 84 (purple in Fig 1, C), with this segment overlapping with the actin-binding site.

The net charge -4 resulting from the 11 basic and 15 acidic amino acids in the 131 residues of Cuc m 2 gave rise to a dominant negative PB electrostatic potential (Fig 1, D) on most of the surface, except at some regions that show small strongly positive (blue) domains amid largely neutral (white) areas.

Sequential IgE-binding epitopes of Cuc m 2 mainly overlap the actin-binding site

Recombinant and natural melon profilin Cuc m 2 were purified, and their IgE-binding capacities were proved by using a pool of sera from patients with melon allergy (Fig 2, A). Furthermore, all 10 individual sera comprising the serum pool exhibited significant levels of specific IgE toward both forms of Cuc m 2 (Fig 2, B).

SPOTs with 10 mers synthetic peptides overlapping 5 amino acids and spanning the entire Cuc m 2 sequence were analyzed by using the serum pool and 4 individual

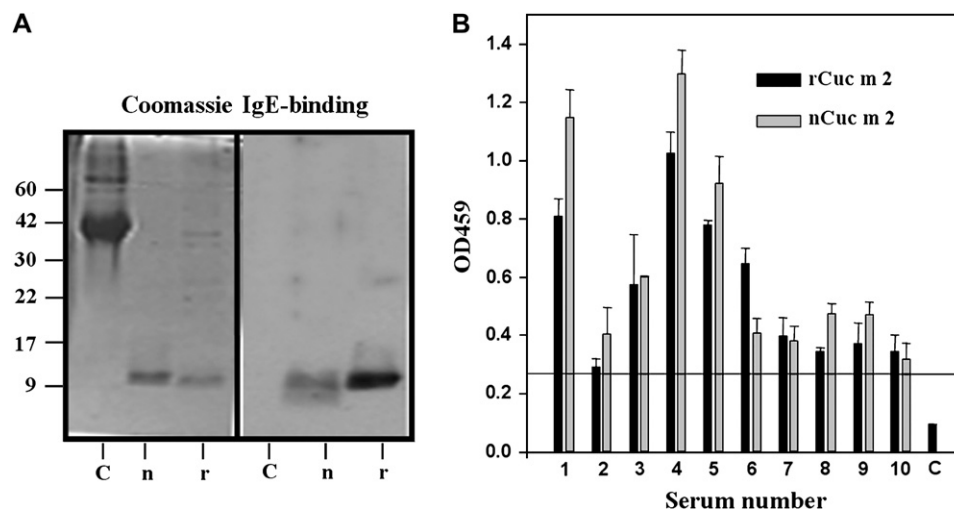


FIG 2. A, Protein staining and IgE immunodetection with sera from patients with melon allergy of purified natural (*n*) and recombinant (*r*) Cuc m 2 and ovalbumin (negative control; C). **B**, Specific IgE of 10 individual sera. BSA (C) was tested as a negative control. Means ($n = 3$) and SDs (bars) are represented. OD values of greater than 0.24 units (3×0.081 of the mean [$n = 7$] obtained for the negative control) were considered positive results.

sera (Fig 3) to identify sequential regions of the allergen involved in IgE binding. Six of these regions were recognized by the serum pool but with very different intensities (Fig 3, A). The main responses were observed for peptides 17 to 19 (positions 81-100), with peptides 17 and 18 also clearly detected by the individual sera 1, 3, and 5. Therefore residues 81 to 95, probably extended to residue 100, seemed to conform to a relevant IgE epitope that overlapped with the actin-binding site of Cuc m 2 (see above).

Two additional regions represented by peptides 14 (positions 66-75) and 26 (positions 122-131) were strongly reactive to the serum pool and serum 5, respectively, pointing to potential relevant IgE epitopes, at least in some patients. Finally, a weak response to the serum pool was found for peptides 2 to 3 (positions 6-20), 8 to 10 (positions 36-55), and 22 to 23 (positions 106-120). Serum 4 did not detect any peptide, despite its high specific IgE levels to natural and recombinant Cuc m 2 (Fig 2, A), suggesting a strict recognition of conformational epitopes.

Converging experimental and model-based derived data uncover 2 main IgE epitopes around the Cuc m 2 actin-binding site

All the residues corresponding to synthetic peptides that showed the main IgE-binding capacity were located on the surface of Cuc m 2, along a middle patch around the actin-binding site (Fig 4, A-C). Residues 81 to 100 (peptides 17-19, blue in Fig 4) were in strands $\beta 5$ and $\beta 6$ and loops $\beta 5\beta 6$ and $\beta 6\beta 7$ (Fig 4, A). Except for the completely buried V94 and A100, these residues formed an elongated surface of $700 \text{ \AA}^2$ (the whole protein has a surface area of $6176 \text{ \AA}^2$). Residues 66 to 75 (peptide 14, green in Fig 4), located in strands $\beta 3$ and $\beta 4$ and loop $\beta 3\beta 4$ (Fig

4, A), made up a convex compact surface of $543 \text{ \AA}^2$ with highly exposed protrusions. Residues 122 to 131 (peptide 26, magenta in Fig 4) spanned half of helix H3 and the C terminus (Fig 4, A) and were located on a surface with the largest area ($711 \text{ \AA}^2$) of all these 3 sequential segments. These surfaces overlapped to a different extent with the actin-binding site and encompass the plant-specific binding pocket (Fig 4, B and C). However, instead of 3 distinct epitopes, these surfaces suggested 2 regions that mapped separately on the allergen and were connected only by a narrow, relatively buried patch, as is readily seen in Fig 4, B. The first region comprised sequence segments 66 to 75 and 81 to 93 (altogether 23 residues), covered an area of $1028 \text{ \AA}^2$, and is hereafter denoted as epitope E1. The second region included segments 95 to 99 and 122 to 131 (a total of 15 residues) with an area of $926 \text{ \AA}^2$ and is accordingly denoted as epitope E2. The actin-binding site was composed of 25 residues with an area of $1495 \text{ \AA}^2$, and although its overlap with E1 amounted to $719 \text{ \AA}^2$ (approximately 72% of the E1 surface), its overlap with E2 was only $115 \text{ \AA}^2$ (approximately 12% of the E2 surface).

The electrostatic properties of epitopes E1 and E2 highlighted their IgE-binding capacity, as well as their differences (Fig 5). The PB isocontours (Fig 5, B) indicate the spatial extent of the electrostatic field created by Cuc m 2. The E1 region was associated with the wide electropositive domain that is nearly coincident with its own protein surface, whereas by contrast, the E2 region lay behind the large electronegative domain that protrudes far beyond the protein surface. These field domains reflected the completely different electrostatic nature of both epitope surfaces, which are connected by a narrow neutral (zero potential) patch (Fig 5, C). E1 was a neutral-positive region (Fig 5, D) and showed a rather high proportion (65%) of hydrophobic residues. On the contrary, E2 was

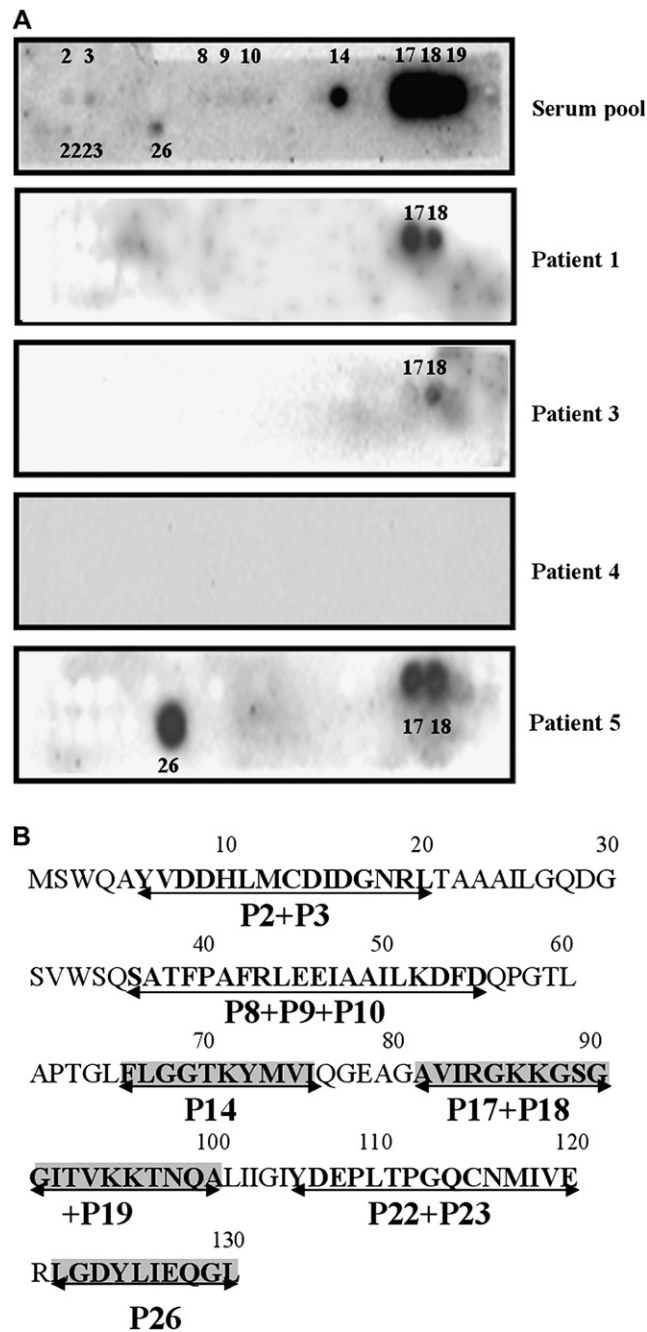


FIG 3. A, IgE immunodetection of SPOTs with synthetic peptides spanning the entire Cuc m 2 amino acid sequence by using a serum pool and 4 individual sera (numbers as in Fig 2, B) from patients with melon allergy. **B**, Amino acid sequence highlighting the positions of major (**bold and shaded**) and minor (**bold**) reactive peptides to the serum pool or the individual sera.

a strongly negative region (Fig 5, E), and its proportion of hydrophobic residues (38%) was lower than that of E1. Basic residues K95 and K96 in E2 were located at the middle right edge of this region, and their effect on the negative potential was thus negligible.

Besides E1 and E2, a joint analysis of prominent electrostatic fields linked to large exposed areas led us to

identify 2 additional theoretical epitopes, hereafter named E3 and E4. The first one included residues 2 to 10 (yellow in Figs 4 and 5), which covered most of helix H1 on a highly exposed and compact protruding surface with an area of 573 Å². E3 partially overlapped with the sequence defined by synthetic peptides 2 to 3 (positions 6-20), which showed a weak reactivity with the serum pool

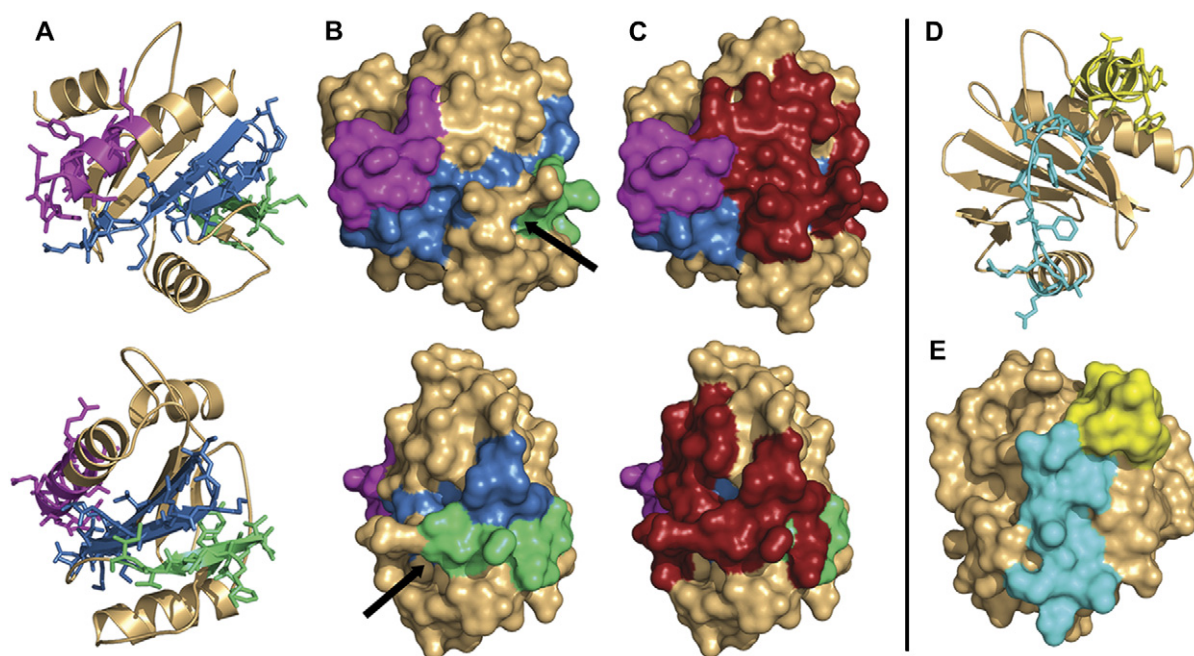


FIG 4. A-C, Epitopes corresponding to peptides 14 (green), 17 to 19 (blue), and 26 (magenta). Fig 4, A, Ribbon diagrams; Fig 4, B, surfaces; and Fig 4, C, overlap with actin-binding site (red). Bottom views of Fig 4, A through C, are derived from upper views by means of a clockwise 90° rotation about a vertical axis (arrows point to the plant-specific pocket). D and E, Ribbon diagram (Fig 4, D) and surface (Fig 4, E) of theoretical epitopes E3 (yellow) and E4 (cyan).

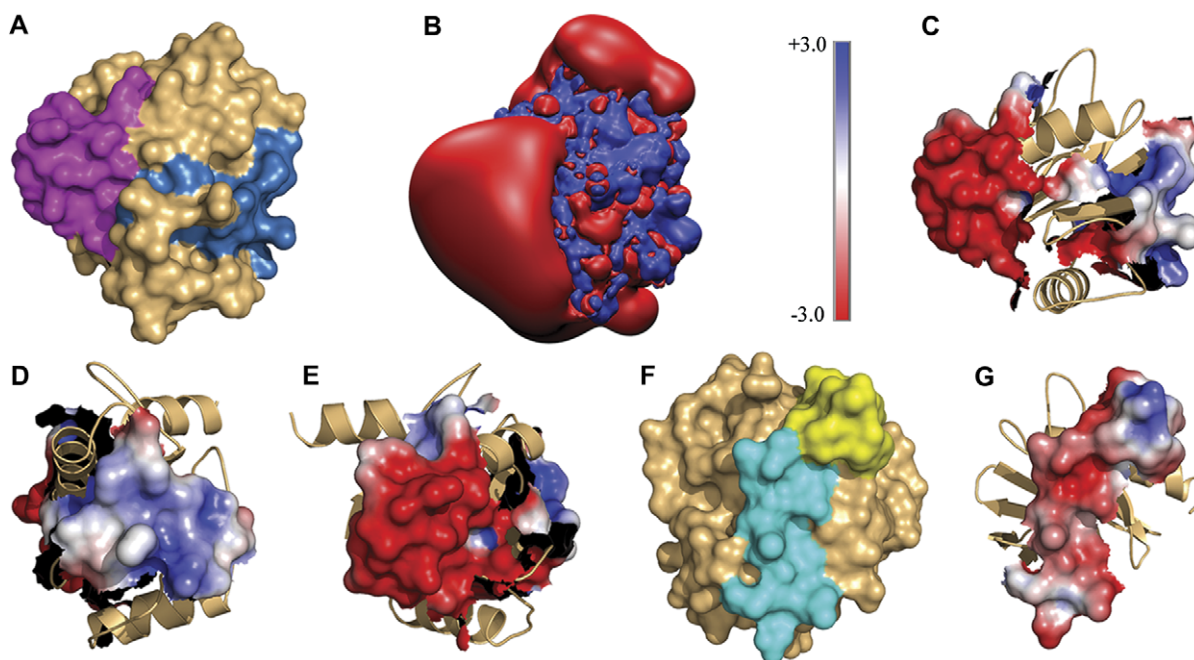


FIG 5. A, Surfaces of epitopes E1 (blue) and E2 (magenta). B, Isocontours -2.0 (red) and $+2.0$ (blue) of PB potential. C, PB potential mapped onto the surface of E1 and E2. D, E1 in front. E, E2 in front. F, Surfaces of E3 (yellow) and E4 (cyan) after a 180° rotation of Fig 5, A, about a vertical axis. G, PB potential.

from patients with melon allergy (Fig 3). The putative epitope E4 extended from residue 35 to 45 (cyan in Figs 4 and 5) in the long coil between strand $\beta 2$ and helix H2 also on a surface with marked protrusions that covered

an area of $694 \text{ \AA}^2$. The sequence fragment represented by the weakly reactive synthetic peptides 8 to 10 (residues 36-55, Fig 3) enclosed E4 (except its first residue 35). The electrostatic properties of epitopes E3 and E4 (Fig 5, F and

G) revealed differences between them, although these were less marked than those between E1 and E2. E3 presented an overall positive character and a low proportion of hydrophobic residues (33%), whereas E4 was predominantly negative and had a higher hydrophobic ratio (55%). A common feature of both E3 and E4 was that the electrostatic potential in their joint surface was much weaker than in the surfaces of either E1 or E2, which suggests *a priori* lower binding energies in the putative antigen-antibody complexes involving the 2 former epitopes.

A third region with low IgE-binding capacity detected by using synthetic peptide analysis, corresponding to peptides 22 to 23 (residues 106-120), showed neither the surface nor electrostatic properties to be considered a potentially relevant epitope.

DISCUSSION

Plant profilins are highly conserved allergens that induce cross-sensitization between a wide variety of sources¹⁻³ recognized by IgE of 10% to 30% of subjects allergic to plant food and pollen.² In contrast to these figures, members of the profilin family from melon (Cuc m 2)³ and orange (Cit s 2)²⁹ are major allergens recognized by more than 70% of patients allergic to these fruits, and almond profilin, Pru du 4, reacts with nearly half of the subjects with almond allergy.³⁰ The high sequence identity of plant profilins (>90% for melon and almond³⁰) supports the relevance of Cuc m 2 as an allergen model to identify epitopes in the search for further information on cross-reactive interactions with IgE. However, insofar as other reports have also shown only partial cross-reactivity^{12,13} and even fine IgE specificity to variable epitopes,¹⁵ one also might consider species-specific epitopes on plant profilins.

The analysis of IgE binding of synthetic peptides covering the whole amino acid sequence of Cuc m 2 together with solvent exposure data and electrostatic potentials computed on the surface of a model structure permitted us to identify 4 possible epitopes of melon profilin. Two of them, denoted E1 and E2, correspond to peptides that show the strongest IgE-binding activity, whereas the other 2, denoted E3 and E4, were predicted on theoretical grounds, although they happen to overlap with peptides that exhibit weak activity. To assess the reliability of our theoretical analysis, one should recall that epitopes occupy large irregular surface areas of approximately 1000 Å² comprised of 15 to 20 residues with protrusions and valleys.^{17,18,31} Besides promoting orientation of the molecules, long-range electrostatic interactions play a major role in stabilizing the complex through the formation of specific contacts in the final docking.^{17,18,31,32} It was proposed that high-affinity antibody-antigen complexes have stronger electrostatic interactions and a lower proportion of hydrophobic residues, whereas a cross-reactive behavior involves less specific contacts (ie, weaker electrostatic interactions and higher hydrophobic ratios).¹⁸

The overall negative electrostatic potential on the surface of Cuc m 2 (Fig 1, D) and the greater extent of

the negative isopotential field compared with the positive equivalent isovalue (Fig 5, B) are consistent with the dominance of acidic amino acids in this profilin. However, the differences between the major epitopes E1 and E2 with regard to their associated field domains and electrostatic nature suggest a distinct behavior. The surfaces of both E1 and E2 display electrostatic characteristics that point to strong ionic antibody-antigen interactions: we must highlight that the -3 to +3 scale used represents considerable energy (double the thermal energy). However, although E1 is neutral-positive and has a high proportion of hydrophobic residues (0.652), E2 is negative and has a lower hydrophobic ratio (0.375). These results suggest that E1 would be a cross-reactive epitope, a suggestion further supported by the fact that three quarters of its surface overlap with the highly conserved actin-binding site. Moreover, irrespective of its antigenic activity, the strong electropositive character of E1 (Fig 5, D) might be related to the interaction with actin, which is known to occur at the negative polar end of actin.²⁸ Similarly, the electronegative E2 would be a high-affinity epitope. It is interesting to recall that a number of antigenic sites and many haptens known to bind strongly to antibodies were earlier observed to exhibit significant negative potentials and large electrostatic fields, with energies greater than thermal energy extending far beyond the surface.³¹ In the case of epitopes E3 and E4, their electrostatic nature points to smaller ionic interactions (ie, weaker antigen-antibody associations in both cases), whereas their hydrophobic ratios (0.333 and 0.545, respectively) suggest that E3 would be a high-affinity epitope and E4 would preferably show cross-reacting behavior.

It must be stressed that our results are in fair agreement with the available evidence on plant profilin epitopes. The actin-binding site and the adjacent plant-specific pocket were found to compose a major immunogenic region responsible for cross-reacting response in *Arabidopsis* profilin.⁸ Two regions overlapping with the actin-binding site were identified as major cross-reactive epitopes, and a third site consisting of residues 30 to 50 was found to be a likely cause of extensive, albeit weaker, cross-reactivity in birch profilin.⁹ Three epitopes at the C-terminal helix (residues 100-109, 108-115, and 122-127) overlapping with the actin-binding site, one epitope at the N-terminal helix (residues 1-15) overlapping with the PLP-binding site, and another epitope formed by segments 40 to 50 were found in sunflower profilin.¹¹ Among the several epitopes (that indeed cover most of the surface) identified in model structures of several profilins, Radauer et al¹³ highlighted 3 major candidates that happen to include a great part of our epitope E2, as well as residues 2 to 10 (just our epitope E3). Leitner et al³³ found that the circular peptide CAISGGYPVC inhibited IgE binding to mugwort pollen, birch pollen, and celery tuber profilin and speculated that this mimotope might represent an important epitope on plant profilins, although they were not able to locate a corresponding area on the crystal structure of the birch profilin Bet v 2. By applying our approach to Bet v 2, we identified a surface region composed of residues

A63-I77-S91-G92-G90-Y74-P64-V76-M75 (1CQA numbering, first cysteine excluded) located at the actin-binding site and adjacent to the plant-specific pocket (results not shown).

We finally emphasize on one side the novelty of our approach to identify potential epitopes and their possible role and on the other side the agreement of our proposals with findings previously reported in the literature on plant profilins obtained on the basis of different methodologies. Furthermore, our data support the use of a representative profilin (ie, Cuc m 2) as a tool for diagnostic purposes and highlight the potential of synthetic peptides to differentiate between specific and cross-reactive sensitization to plant profilins.

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