

Hev b 8, the *Hevea brasiliensis* Latex Profilin, Is a Cross-Reactive Allergen of Latex, Plant Foods and Pollen

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Key Words

Recombinant Hev b 8 · Profilin · Latex allergy · *Hevea brasiliensis* · Cross-reactivity

Abstract

Background: Plant profilins are important pan-allergens. They are responsible for a significant percentage of pollen-related allergies. Limited information is available about their involvement in the latex-fruit syndrome and the cross-reactivities between latex and pollen. We aimed to clone and express the *Hevea brasiliensis* latex profilin to investigate its allergological significance and serological cross-reactivities to profilins from plant foods and pollens. **Methods:** A DNA complementary to messenger RNA (cDNA) coding for the *Hevea* latex profilin, Hev b 8, was amplified by polymerase chain reaction from latex RNA. Recombinant (r)Hev b 8 was produced in *Escherichia coli* and used to screen sera from 50 latex-

allergic health care workers (HCWs) with well-documented histories of food and pollen allergy and 34 latex-allergic spina bifida (SB) patients. The cross-reactivity of natural Hev b 8 and rHev b 8 with other plant profilins was determined by ELISA inhibition assays. A three-dimensional homology model of Hev b 8 was constructed based on known profilin structures. **Results:** The cDNA of Hev b 8 encoded a protein of 131 amino acids with a predicted molecular mass of 14 kD. Twelve of the 50 HCWs and 2 of the 34 SB patients were sensitized to Hev b 8. All Hev b 8-sensitized patients showed allergic symptoms to pollen or plant foods. Cross-reactivities between profilins of latex, pollen and plant food were illustrated by their ability to inhibit IgE binding to rHev b 8. Homology modeling of Hev b 8 yielded a structure highly similar to Bet v 2, the birch pollen profilin, with the most distinct differences located at the N-terminus. **Conclusions:** We conclude that primary sensitization to latex profilin in the majority of cases takes place via pollen or food profilins. Additionally, pollinosis and food-allergic patients with profilin-specific IgE can be at risk of developing latex allergy.

The complete cDNA sequence of Hev b 8.0204 is available from the EMBL database under the accession number AJ243325.

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Introduction

Type I latex allergy has become an important health problem that is increasing both in its frequency and in the severity of its clinical manifestations. Considerable effort has been undertaken to characterize the molecular features of *Hevea brasiliensis* latex allergens because of the high prevalence of latex allergy in well-defined risk groups such as health care workers (HCWs), rubber industry workers and children with spina bifida (SB) and urogenital abnormalities [1, 2]. More than a dozen individual latex allergens have been identified, of which eleven have received an international nomenclature designation [3] and (<ftp://ftp.biobase.dk/pub/who-iuis/allergen.list>).

It is well known that a substantial number of patients who are allergic to natural rubber latex also develop allergic reactions to certain plant foods. Cross-reactivity between natural rubber latex and various foods resulting in clinical manifestations has been designated as the latex-fruit syndrome [4–6]. Structural similarities between *Hevea* allergens and food allergens are responsible for the observed clinical and immunochemical cross-reactivities between latex and banana, chestnut, avocado and other fruits.

Class I chitinases have been identified as major pan-allergens in fruits associated with the latex-fruit syndrome, such as avocado [7], banana [8] and chestnut [9]. The conserved N-terminal hevein domain (Hev b 6.02) of the major latex allergen prohevein (Hev b 6.01) is a chitin-binding motif that is also present in class I chitinases and contributes to the observed cross-reactivity between latex and several fruits [10]. β -1,3-Glucanases contribute to the cross-reactivity observed in the latex-fruit syndrome because of the high amino acid sequence similarities among these enzymes from even phylogenetically distant plant species [11]. Lysozymes are present in *Hevea* latex and are also found in plant foods. Structural similarities between latex and fruit lysozymes may account for some of the cross-reactivities seen [12].

Profilins are ubiquitous plant allergens known to cause cross-reactivity between pollen and plant food. The first plant profilin that was identified was the allergen Bet v 2 from birch pollen [13]. It has been shown that profilins are involved in different clinical cross-reactivity syndromes, such as the celery-mugwort-spice syndrome [14]. Furthermore, IgE of pollen-allergic individuals cross-reacts with profilins from apple [15], bell pepper [16], celery [17], lychee [18], peanut [19], pumpkin seed [20], soybean [21] and tomato [22]. This widespread IgE cross-reactivity towards a broad range of profilins from distantly related

plants has led to the designation of profilins as pan-allergens. Profilins are low-molecular-weight proteins found in all eukaryotic cells, where they function as actin-binding proteins involved in the regulation of the actin cytoskeleton [23]. They are highly conserved proteins, and sequence comparisons among several plant profilins show an identity of at least 75%. In the past few years, profilins have been identified as allergens in pollen of grasses, weeds and trees and in many fruits and vegetables of numerous plant species [24]. Patients sensitized to profilins characteristically react to a broad range of inhalant and nutritive allergen sources.

The profilin of *H. brasiliensis* latex was first identified as an allergen by IgE inhibition assays [25]. Sequences of DNAs complementary to messenger RNA (cDNAs) of several *H. brasiliensis* profilins, designated Hev b 8 by the international allergen nomenclature committee, are available from the EMBL/GenBank database. Beezhold et al. identified three highly similar profilin sequences from *H. brasiliensis* latex (accession numbers AF119365, AF119366 and AF119367). In addition, Asturias et al. cloned a profilin sequence from *H. brasiliensis* pollen (AJ132397), and Rihs et al. amplified a profilin sequence from RNA isolated from *H. brasiliensis* leaves (AJ132397). Based on the similarities of the reported sequences, *H. brasiliensis* profilins can be divided into two groups, i.e. profilins from the latex-producing laticifers and profilins from other tissues of the plant.

The recent study on the IgE-binding properties of Hev b 8 performed by Rihs et al. [26] used a recombinant *Hevea* profilin whose RNA had been isolated from the leaves of the plant. In the present study, we have produced a recombinant profilin from the latex of *H. brasiliensis* as a nonfusion protein, compared its IgE-binding properties with natural (n)Hev b 8 present in protein extracts from *H. brasiliensis* latex and studied its cross-reactivity with recombinant birch pollen profilin (rBet v 2) and natural profilins from birch pollen (Bet v 2), timothy pollen (Phl p 12) and celery tuber (Api g 4). In addition, we have tested 84 sera of individuals allergic to latex (50 HCWs and 34 SB patients) in regards to their IgE reactivity to rHev b 8, in order to elucidate the role Hev b 8 plays in the primary sensitization to profilins.

Materials and Methods

Isolation of Total RNA from H. brasiliensis Latex

Fresh latex was obtained from regularly tapped Malaysian rubber trees (*H. brasiliensis*; cultivar RRIM 600). Latex exuding from the tapped trees was collected while continuously mixing it with an equal

volume of RNA extraction buffer [0.1 M Tris-HCl, 0.3 M LiCl, 1 mM EDTA, 10% sodium dodecyl sulfate (SDS), pH 9.5] at ambient temperature. The mixture was centrifuged at 100,000 g for 30 min at 15°C. The rubber plug was removed, the aqueous phase extracted with chloroform-phenol and total RNA was precipitated with LiCl.

Reverse Transcription Polymerase Chain Reaction Amplification and Sequencing of the rHev b 8 cDNA

First-strand cDNA synthesis was performed by reverse transcribing 0.5 µg of total latex RNA using the RNA-PCR kit from Applied Biosystems, Langen, Germany, and a modified oligo-d(T) primer, T₂₅NN [5'-GGAGAAGGA(T)₂₅(A/G/C)N-3']. For the polymerase chain reaction (PCR), 2 µl of the first-strand cDNA synthesis were used to amplify part of the coding sequence for Hev b 8. Primers were designed based on the latex profilin sequences deposited in the EMBL database by Beezhold et al. (EMBL accession numbers AF119365, AF119366 and AF119367). The primers used to amplify a partial sequence of the Hev b 8 cDNA were Prof2 (5'-TGGATC-CCTTGCCCCAACT-3') and Prof4 (5'-AGTACATGGTGATCC-AGGGAG-3'). The PCR products were ligated into the pGEM-T Easy vector (Promega, Madison, Wisc., USA) and sequenced using the Thermo Sequenase fluorescent labeled primer cycle sequencing kit (Amersham Pharmacia Biotech, Little Chalfont, UK).

To complete the missing 5' end of the cDNA, the Marathon cDNA Amplification Kit (Clontech, Palo Alto, Calif., USA) was used according to the vendor's instructions. cDNA synthesis using the oligo-d(T) primer from the kit was followed by first- and second-round PCR with the kit's adaptor primers and the gene-specific internal primers Prof1 (5'-TGCCATCGTGGCCGATGATA-3') and Prof3 (5'-AGTTGGGGCAAGGGATCCAG-3'). The PCR products were cloned and sequenced as described above.

The complete cDNA sequence and its deduced amino acid sequence were compared to the EMBL/GenBank and SWISS-PROT databases using the FastA and PileUp programs from the GCG sequence analysis package (Genetics Computer Group, Madison, Wisc., USA).

Homology Modeling

A model of the three-dimensional structure of Hev b 8 was generated using the SWISS-MODEL server (<http://www.expasy.ch/swissmod/SWISS-MODEL.html>) [27]. The model was based on the structures of birch profilin (PDB accession number 1CQA) [28] and the profilin from *Arabidopsis thaliana* (PDB accession number 1A0K) [29] and on birch profilin alone.

Construction of the Expression Plasmid

The complete coding sequence of Hev b 8 was amplified by reverse transcription (RT)-PCR from total latex RNA. The primers used in the PCR were Prof5ex (5'-ACATATGTCGTGGCAACG-TACGTT-3', *NdeI* site underlined) and Prof3ex (5'-AGAATTCAT-TACAGCCTTGATCAAG-3', *EcoRI* site underlined), according to the 5' and 3' end of the coding sequence of the latex Hev b 8 cDNA. The amplification product was digested with *NdeI* and *EcoRI* and ligated into the pMW172 expression vector [30]. The expression construct pMW172/Hev b 8 was transformed into competent *Escherichia coli* BL21[DE3] cells [31].

Expression and Purification of rHev b 8

Cultures for production of rHev b 8 were inoculated to a concentration of approximately 4×10^7 cells/ml (optical density_{600 nm} =

0.1). The medium used for the production of profilin contained 1% peptone, 0.5% yeast extract, 1% NaCl, 10 mM MgSO₄, 0.25% glycerol, 8.5 mM KH₂PO₄ and 36 mM K₂HPO₄, pH 7.4. The growing cells were induced with 0.4 mM isopropyl β-D-thiogalactopyranoside at optical density_{600 nm} = 1.0, incubated for 6 h, harvested and frozen at -20°C.

For purification, cells were thawed at room temperature and suspended in 5 ml/g (wet weight) of 25 mM imidazole, pH 7.4, 10 mg/l phenylmethylsulfonylfluoride, 5 mM β-mercaptoethanol and 0.1% Triton X-100. Cells were lysed by mechanical shearing and two freeze/thaw steps. The extract was cleared by centrifugation (40 min at 40,000 g) and treatment with Biocryl BPA-1000 (TosoHaas, Stuttgart, Germany) and dialyzed against buffer A (25 mM imidazole, pH 7.4, 10 mg/l phenylmethylsulfonylfluoride, 5 mM β-mercaptoethanol and 150 mM NaCl). The dialysate was applied to a poly-L-proline sepharose column. The column was washed with buffer A and buffer A containing 2 M urea and finally the profilin was eluted with 6 M urea in buffer A [32]. Protein-containing fractions were dialyzed against 5 mM phosphate buffer, pH 7.4, and 0.5 mM β-mercaptoethanol and lyophilized.

Protein Extracts

Fresh *H. brasiliensis* latex was collected from rubber trees (cultivar RRIM 600) in chilled containers and centrifuged at 44,000 g at 4°C for 1 h. The aqueous phase, the C-serum, was collected and centrifugation was repeated. The clear aqueous C-serum was freeze dried and stored at -20°C.

Celery (*Apium graveolens*) tuber was purchased from a local retail shop and ground in liquid nitrogen, and proteins were extracted by shaking overnight at 4°C in potassium phosphate buffer. After centrifugation (40,000 g for 60 min at 4°C), the supernatant was filtered, dialyzed and lyophilized. Pollen from birch (*Betula verrucosa*) and timothy grass (*Phleum pratense*) were obtained from Allergon (Ängelholm, Sweden), extracted in a carbonate buffer solution at 4°C for 20 h and centrifuged (23,000 g for 60 min at 4°C). The extract was then dialyzed and lyophilized.

Patients and Patients' Sera

The study population of latex-allergic individuals consisted of 50 HCWs with positive case histories, positive radioallergosorbent tests (RAST class >2) and characteristic type I allergic reactions to latex. In addition, serum samples were obtained from 34 SB patients with latex allergy. Seven serum samples from pollen-allergic individuals with sensitization to pollen profilins from birch, timothy grass and mugwort were used to examine the cross-reactivity of pollen profilin-specific IgE with *Hevea* latex profilin. These seven individuals were selected from patients not suffering from allergic symptoms to latex according to their clinical history. For inhibition experiments, sera from 21 patients containing specific IgE to rHev b 8 were pooled. These serum samples were chosen from among the HCWs (patients 6, 10, 11, 18, 19, 20, 22, 25, 26, 30, 46 and 48), the SB patients (patients 20 and 34) and the pollen-allergic individuals (patients 1-7). A serum pool of 10 nonallergic individuals served as control. The average total IgE of the sera used in the nonallergic control was 49.3 kU/l.

Total serum IgE was determined using the Pharmacia CAP system (Pharmacia-Upjohn, Uppsala, Sweden). Diagnosis of allergy to fruits and vegetables was established by clinical manifestation of symptoms.

SDS-Polyacrylamide Gel Electrophoresis and Immunoblotting

Protein extracts (50 µg per lane), rHev b 8 (1 µg per lane) and rBet v 2 (1 µg per lane) were separated by 12% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions and transferred to Protran nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany) for immunodetection. Immunoblots were performed using 50 mM potassium phosphate, 0.05% Tween 20, 1% powdered milk for blocking, washing and antibody dilutions. Blotted proteins were incubated with sera from patients (diluted 1/5). Bound IgE was detected using ¹²⁵I-labeled rabbit anti-human IgE (IBL, Hamburg, Germany), diluted 1/20. Alternatively, a polyclonal monospecific rabbit anti-celery profilin antibody (kindly provided by Dr. Philippe Deviller, Lyon, France) was used (dilution 1/1,000). Bound rabbit antibody was detected by ¹²⁵I-labeled donkey anti-rabbit antibody (Amersham Pharmacia Biotech), diluted 1/1,000. Bound immunoglobulins were visualized on Biomax MS film (Kodak, Rochester, N.Y., USA).

For inhibition of IgE binding to nHev b 8, nBet v 2, nPhl p 12 and nApi g 4, a pool of Hev b 8-positive sera was incubated overnight at 4°C with 100 µg of purified rHev b 8. The preincubated sera were then used to probe nitrocellulose strips containing latex C-serum and protein extracts from birch pollen, timothy pollen and celery tuber. Incubation of the serum pool with 100 µg of purified rHev b 3 was used as a negative control.

IgE Inhibition ELISA

For cross-inhibition experiments, a profilin-specific serum pool consisting of 21 sera with IgE reactivity to rHev b 8 was diluted 1/5 in Tris-buffered saline (TBS), 0.05% Tween 20 and 0.5% BSA. This serum pool was preincubated (overnight at 4°C) with 0.013, 0.025, 0.05, 0.1, 0.5, 1 and 1.5 µg/ml rHev b 8, rBet v 2 or rHev b 3 as negative control. In addition, the serum pool was preincubated with 0.5, 1, 5 or 10 µg of protein extracts from the *Hevea* latex C-fraction, birch pollen, celery tuber, timothy pollen or BSA as negative control. Microtiter plates (Nunc-Immuno Plate, Nunc, Roskilde, Denmark) were coated with 4 µg/ml of recombinant allergens overnight at 4°C. After blocking with TBS, 0.05% Tween 20 and 1% BSA, preincubated sera were applied onto the coated plates and incubated overnight at 4°C. After washing five times with TBS and 0.05% Tween 20, the plates were incubated with alkaline phosphatase-conjugated mouse anti-human IgE antibody (Pharmingen, San Diego, Calif., USA) for 1 h at 37°C and for another hour at 4°C.

Color development was performed using phosphatase substrate tablets [each containing 5 mg of *p*-nitrophenyl phosphate disodium (Sigma-Aldrich, Steinheim, Germany)] according to the vendor's instructions. The optical density was measured at 405 nm (550 nm as reference wavelength) after 30 min.

Results

Cloning and Sequence Analysis of Hev b 8

Primers designed according to the sequences deposited in the EMBL/GenBank database by Beezhold et al. were used to amplify most of the coding region and the complete 3' end of a *Hevea* latex Hev b 8 cDNA by RT-PCR from total latex RNA. The cDNA was completed at the 5' end using the Marathon cDNA Amplification Kit. The

complete cDNA of Hev b 8 comprised 824 base pairs, including a poly(A) tail of 24 adenosyl residues. The cDNA sequence contained an open reading frame coding for 131 amino acids (fig. 1) with a predicted molecular weight of 14.0 kD and a theoretical isoelectric point of 4.87. It is available from the EMBL database under the accession number AJ243325. The allergen was assigned the designation Hev b 8.0204 by the WHO IUIS allergen nomenclature subcommittee (<ftp://ftp.biobase.dk/pub/who-iuis/allergen.list>).

The deduced amino acid sequence of Hev b 8 was compared to other sequences in the EMBL and SWISS-PROT databases. The protein displayed between 95 and 98% identity with the other latex profilin isoform sequences from *Hevea* cultivar RRIM 600 deposited in the database by Beezhold et al., but only 83% identity with a pollen profilin from *Hevea* cultivar RRIC 133 and 81% identity with the profilin sequence deduced from a cDNA clone that had been amplified from *Hevea* cultivar RRIM 600 leaf RNA [26]. Sequence identities with allergenic profilins from other plants are listed in table 1. A sequence alignment of the *Hevea* latex profilin to the *Hevea* leaf profilin (80.9% identity) and selected pollen and food profilins is shown in figure 1.

Homology Modeling

Homology modeling of the three-dimensional structure of Hev b 8 yielded a conformation that was highly similar to the structures of the templates, the profilins from birch and *Arabidopsis* (fig. 2a). The three proteins differ only in the orientation of the N-terminal α-helix (α1) and the conformation of the adjoining loop. Since the loop has no defined conformation in the Bet v 2 structure, the model for this part of the Hev b 8 molecule is based solely on the structure of the *Arabidopsis* profilin. The orientation of α1, which is influenced by the loop conformation, consequently resembles the *Arabidopsis* profilin structure. A second model was generated using only Bet v 2 as the template, resulting in a structure nearly identical to Bet v 2 (data not shown). Thus, the structure of the N-terminal part of Hev b 8 cannot be reliably predicted. Localization of the major linear IgE epitopes of Bet v 2 [28] on the Hev b 8 model showed that all three epitopes are situated on the surface of the protein (fig. 2b).

Expression and Purification of rHev b 8

The coding region of Hev b 8 was inserted into the expression vector pMW172, and rHev b 8 was produced as a nonfusion protein in transformed *E. coli* BL21[DE3] cells. The recombinant protein was purified by chroma-

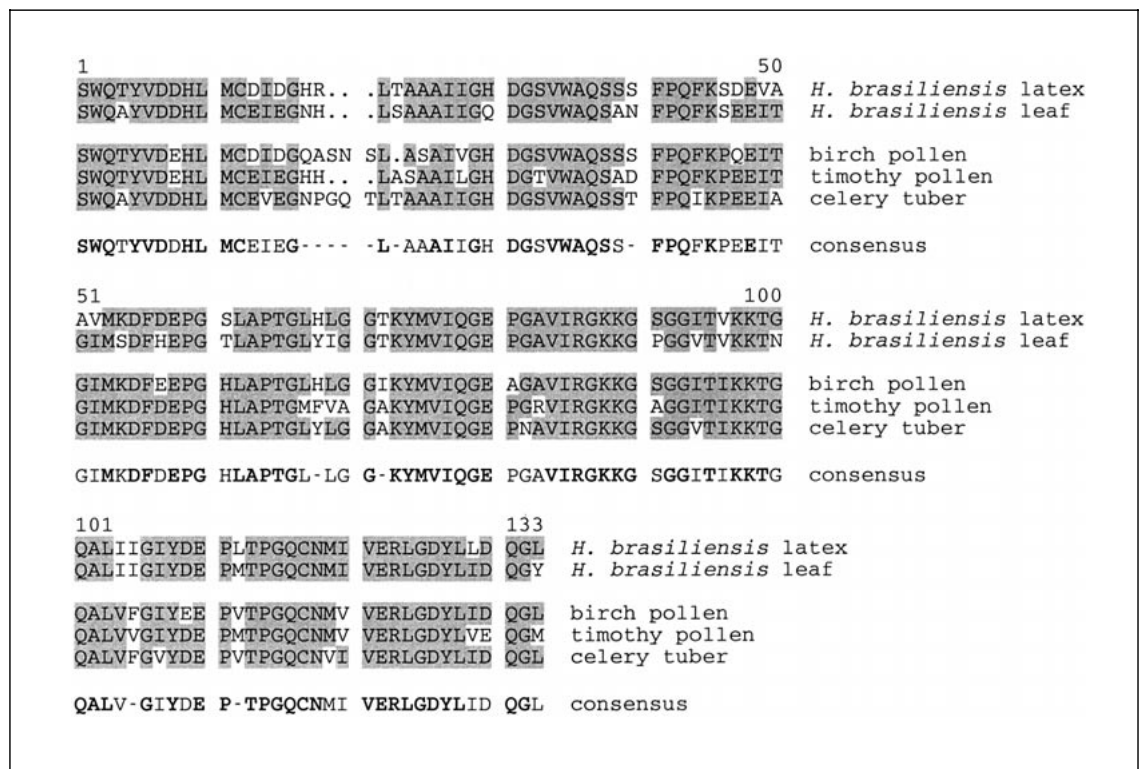


Fig. 1. Multiple protein sequence alignment of profilins from different plant species (*H. brasiliensis*, *B. verrucosa*, *P. pratense* and *A. graveolens*). Pairwise alignments revealed that Hev b 8 displays 81% sequence identity to an isoform amplified from *H. brasiliensis* leaves (EMBL/GenBank accession number AJ132397), 82% sequence identity to Bet v 2 (SWISS-PROT accession number P25816), 76% to Phl p 12 (SWISS-PROT accession number P35079) and 81% to Api g 4 (EMBL/GenBank accession number AF129423). The residues corresponding to the consensus sequence are shaded. Residues identical in the shown profilin sequences are in bold in the consensus sequence.

Table 1. Sequence comparison of Hev b 8 with plant profilins characterized as allergens

Allergen source (accession number)	Similarity %	Identity %
<i>Plant pollen</i>		
Ole e 2/olive (Y12425) [39]	93.9	82.4
Bet v 2/birch (M65179) [13]	93.9	81.7
Cyn d 12/bermuda grass (Y08390) [40]	91.6	80.2
Mer a 1/ <i>Mercurialis annua</i> (Y13271) [36]	93.9	78.6
Phl p 12/timothy grass (X77583) [41]	90.8	75.6
Hel a 2/sunflower (Y15210) [42]	89.3	71.8
<i>Plant food</i>		
Pyr c 4/pear (AF129424)	95.5	87.1
Pru av 4/sweet cherry (AF129425)	93.2	84.1
Api g 4/celery (AF129423) [17]	93.9	81.1
Gly m 3/soybean (AJ223982) [21]	90.8	80.2
Ara h 5/peanut (AF059616) [19]	88.6	76.5

tography using a poly-*L*-proline sepharose affinity column. Elution from the column yielded a product migrating at 14 kD that was free of contaminating *E. coli* proteins as determined by a silver-stained SDS-PAGE gel (data not shown). The average yield of purified rHev b 8 was 90 mg/l of bacterial culture.

Patients' Characteristics

Clinical data of the 50 latex-allergic HCWs (41 females, 9 males) are summarized in table 2. Thirty-one (62%) of these patients showed allergic symptoms to at least one plant food. Twenty-two (44%) patients suffered from allergic symptoms to birch, grass or mugwort pollen. Fifteen (30%) of the latex-allergic patients were allergic to fruits and/or vegetables without associated allergy to pollen. Six patients (12%) were allergic to birch, grass or mugwort pollen but suffered from no plant food allergy.

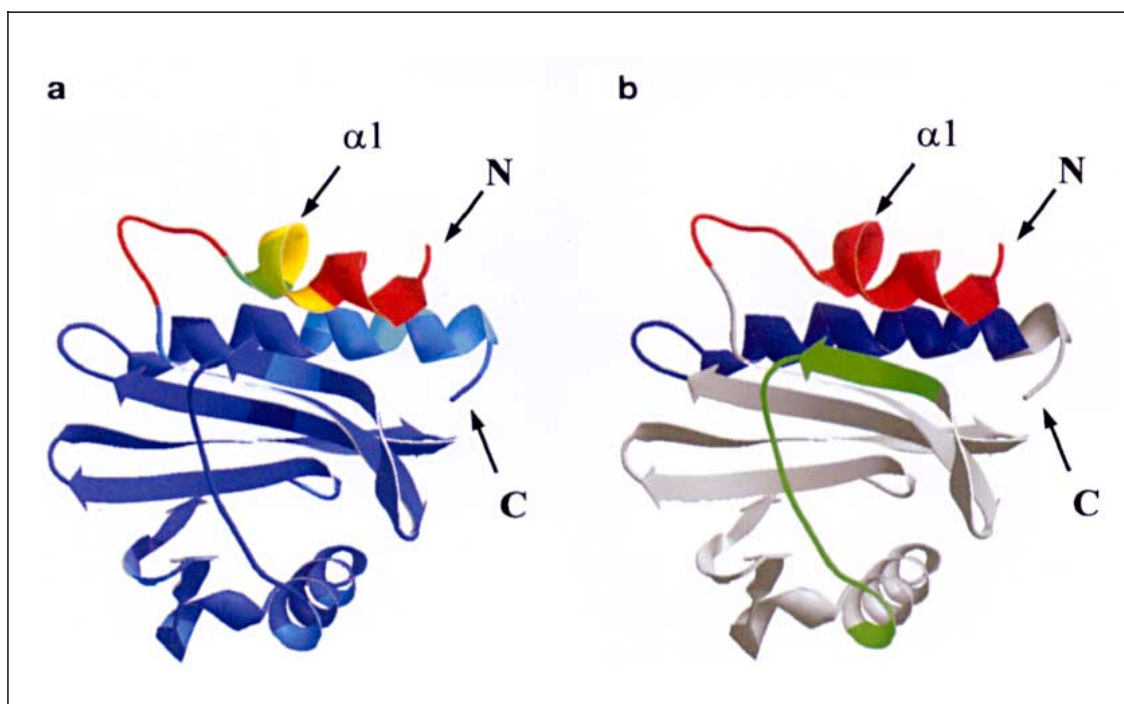


Fig. 2. Model of the three-dimensional structure of Hev b 8 based on the structures of Bet v 2 and the *A. thaliana* profilin. Only the backbone with its secondary structure is shown. N = N-terminus; C = C-terminus; $\alpha 1$ = N-terminal α -helix. **a** Deviations from the Bet v 2 structure. The root mean square distance between corresponding backbone atoms is color coded from dark blue (lowest) to red (highest). **b** Linear IgE epitopes determined for Bet v 2 localized on the Hev b 8 structure. The epitopes comprise the N-terminal α -helix $\alpha 1$ (red), β -strand 2, a loop and part of the α -helix $\alpha 2$ (green) and the C-terminal α -helix $\alpha 3$ (blue). The figures were generated using the Swiss-Pdb Viewer [27].

Twelve (24%) of the 50 HCWs were sensitized to Hev b 8. All these individuals showed allergic symptoms to food and/or pollen. The frequency of Hev b 8 sensitization was 32% (10 out of 31) among food-allergic HCWs, 36% (8 out of 22) among pollen-allergic HCWs, 32% (12 out of 37) among individuals allergic to food and/or pollen and 38% (6 out of 16) among HCWs allergic to allergen sources from both groups. None of the 13 latex-allergic patients without an associated pollen or food allergy possessed rHev b 8-specific IgE.

The screening of 34 serum samples of latex-allergic SB patients (25 males, 9 females) for IgE reactivity to rHev b 8 resulted in the identification of 2 (6%) positive sera. Both patients were male and showed a food or pollen allergy. One patient had 14 kU/l latex-specific IgE (RAST class 3), while the other had 24 kU/l latex-specific IgE (RAST class 4).

All seven grass- and birch pollen-allergic individuals with IgE specific for pollen profilins of birch, timothy

grass and mugwort but without latex allergy also recognized rHev b 8 in immunoblot experiments. All these patients showed allergic symptoms to at least one plant food (table 3). Likewise, IgE from all rHev b 8-positive latex-allergic patients recognized rBet v 2 and a 14-kD band in blotted extracts from the latex C-fraction, birch pollen, timothy pollen and celery tuber.

Immunoblot Studies with r Hev b 8

Protein extracts from *H. brasiliensis* latex, birch pollen, timothy pollen and celery tuber were blotted to nitrocellulose. Profilins were detected by an anti-celery profilin antibody (fig. 3, lanes a). A serum pool of 21 rHev b 8-positive sera was used to assess IgE binding to profilins in the protein extracts (fig. 3, lanes b). At a concentration of 100 μ g/ml rHev b 8, IgE binding to all profilins was completely abolished (fig. 3, lanes c). No inhibition was observed when the serum pool was preincubated with 100 μ g/ml rHev b 3 (fig. 3, lanes d).

Table 2. Characterization of the study population of 50 latex-allergic HCWs

Patient No.	Sex	Total IgE kU/l	Latex RAST class	IgE to rHev b 8	Pollen allergy	Plant food allergy
1	F	105	3		+	+
2	F	114	2			+
3	F	384	3			
4	F	156	2		+	
5	F	139	5			
6	F	342	3	+	+	
7	F	473	3		+	+
8	F	>2,000	3			
9	F	115	4		+	+
10	M	428	2	+		+
11	M	72	2	+	+	+
12	F	342	3		+	
13	F	1,401	3			
14	F	118	5		+	+
15	F	>2,000	3		+	+
16	F	107	4		+	
17	M	262	5			+
18	F	214	3	+		+
19	F	616	5	+	+	+
20	M	276	3	+	+	+
21	M	276	3			
22	F	1,738	3	+	+	
23	F	418	3		+	+
24	F	164	3			
25	F	273	3	+		+
26	M	833	3	+		+
27	F	982	2		+	+
28	M	253	2			+
29	F	125	2		+	+
30	F	2,000	5	+	+	+
31	F	139	2			
32	M	96	2		+	+
33	F	210	4			+
34	F	2,000	5			+
35	F	922	2			+
36	F	38	3			
37	F	31	2			
38	F	67	2			
39	F	50	3			
40	F	368	3			
41	F	138	4			+
42	F	67	5			+
43	F	40	5			+
44	F	133	5			+
45	F	2,000	3		+	+
46	M	181	3	+	+	+
47	F	259	2			
48	F	271	3	+	+	+
49	F	2,000	3			+
50	F	16	3		+	

Table 3. Characterization of pollen profilin-allergic patients without latex allergy

Patient No.	Sex	Total IgE kU/l	RAST class for Bet v 2	IgE to rHev b 8	Pollen allergy	Plant food allergy
1	M	80.6	1	+	+	+
2	M	1,349	6	+	+	+
3	F	194	4	+	+	+
4	F	>2,000	5	+	+	+
5	M	334	3	+	+	+
6	M	133	3	+	+	+
7	F	203	1	+	+	+

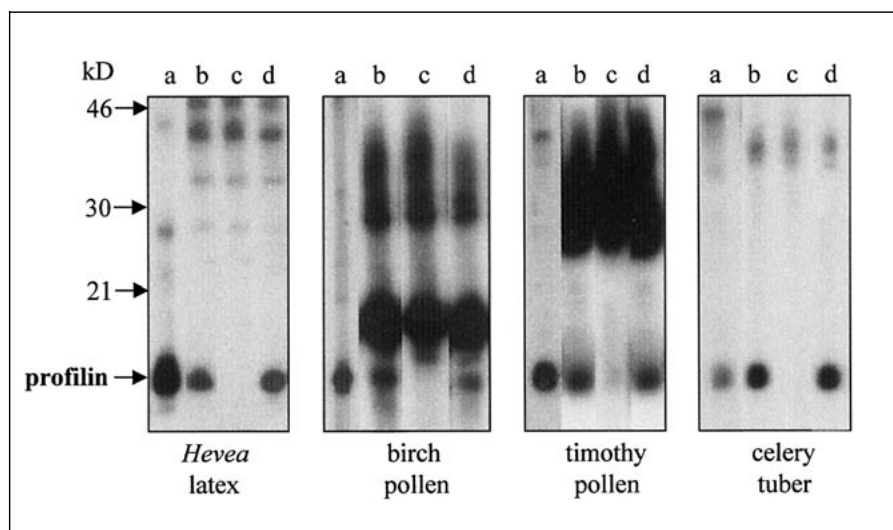
ELISA Inhibition Studies with rHev b 8 and rBet v 2

For cross-inhibition studies, 21 sera from allergic patients displaying IgE reactivity to rHev b 8 were used as a serum pool in ELISA experiments. The two recombinant profilins Bet v 2 and Hev b 8 were coated at a concentration of 4 µg/ml and tested with aliquots of the serum pool after preincubation with increasing amounts of rHev b 8 and rBet v 2. rHev b 8 was able to inhibit IgE binding to rBet v 2 coated to the solid phase at a concentration of 0.2 µg/ml (fig. 4a), and rBet v 2 abolished IgE binding to solid phase-coated rHev b 8 at the same concentration (fig. 4b). These results indicate a high degree of structural identity between the two molecules. As positive controls, inhibition of IgE binding was tested with the autologous molecules (Bet v 2 inhibited by Bet v 2 and Hev b 8 inhibited by Hev b 8), resulting in comparable curves to those achieved with the homologous molecules (fig. 4a, b). For negative controls, the serum pool was preincubated with equivalent amounts of purified rHev b 3, which had no influence on the IgE binding to recombinant profilins (fig. 4a, b).

ELISA Inhibition Studies with Pollen, Latex and Celery Protein Extracts

rHev b 8 was coated to the solid phase. Protein extracts containing the endogenous profilins of the latex C-fraction, birch pollen, celery tuber and timothy grass (*P. pratense*) were used for the preincubation of the serum pool. The profilins present in these extracts were all able to inhibit IgE binding to rHev b 8 in a dose-dependent manner. The profilin content of the *P. pratense* pollen protein extract was the highest of all extracts tested. Inhibition of patients' rHev b 8-specific IgE was achieved at 0.5 µg of protein/ml (fig. 5). In contrast, the nHev b 8 content in the latex C-fraction was approximately 20-fold lower than in

Fig. 3. Immunoblot determination of the cross-reactivity of Hev b 8. Protein extracts from *Hevea* latex, birch pollen, timothy pollen and celery tuber were separated by 15% SDS-PAGE and transferred to nitrocellulose membranes. Blot strips were incubated with the following antibodies: rabbit anti-celery profilin (lanes a); pool of allergic patients' sera containing rHev b 8-specific IgE (lanes b); serum pool as in lanes b preincubated with 100 µg/ml rHev b 8 (lanes c); serum pool as in lanes b preincubated with 100 µg/ml rHev b 3 (lanes d).



the *P. pratense* extract, resulting in inhibition of the IgE binding at a concentration of 10 µg of protein/ml (fig. 5). The inhibition curves obtained by preincubation of the serum pool with birch pollen and celery tuber protein extracts also show complete inhibition at a concentration of 10 µg of protein/ml. BSA had no influence on IgE binding (fig. 5).

Discussion

It is a well-known fact that an association between latex allergy and hypersensitivity to foods exists [5, 6]. A study from the Canary Islands found that 52% of latex-allergic patients had allergies to certain fruits [5]. Another study performed in Münster, Germany, reported that 42.5% of their latex-allergic patients had symptoms after ingestion of fruits [6]. In the present study, 62% of the latex-allergic HCWs showed allergic symptoms to at least one plant food (table 2). The differences between the values found in these three studies may be due to differences in nutritional habits or to the steady increase in allergies in general. In addition, we determined a 44% prevalence of pollen allergy within our study group.

Profilin in plants was first isolated from birch pollen and described as the allergen Bet v 2 due to its ability to bind specific IgE from birch pollen-allergic individuals [13]. Because of high sequence similarities between profilins, and consequently a comparable immunogenicity, profilins have been described as pan-allergens of various plant species [33]. The frequency of IgE reactivity to Bet

v 2 has been reported to be about 20% in birch pollen-allergic individuals [33, 34]. IgE reactivity to olive pollen profilin has been reported to be 24% [35], to the profilin of the *Euphorbiaceae* plant *Mercurialis annua* 59% [36] and to the profilin of soybean 69% [21]. All these data indicate the high significance of profilins as elicitors of IgE-mediated allergies. Thus, it was of particular interest that profilin was also identified as an allergen in *Hevea* latex by IgE inhibition experiments [25]. A possible cross-reactivity with its homologues in plant foods and pollen was inferred from CAP inhibition assays using sera from fruit- and vegetable-allergic patients [37].

In the present study, we report the cloning of a *H. brasiliensis* latex profilin cDNA. Comparison to the previously published *Hevea* profilin sequences showed high identities with the other profilin isoforms from *Hevea* latex (94.7–97.7%), but a comparably large divergence from the profilin sequences obtained from *Hevea* pollen (83.2% identity) or leaf RNA (80.9% identity). Sequence identities of around 80% between the latex and pollen or leaf profilins of *H. brasiliensis* are in the same range as, for example, identities between the latex profilin and profilins from birch, Bermuda grass and soybean (table 1). These findings demonstrate that there are two groups of profilins in *H. brasiliensis*, profilins from the cytoplasm of the highly specialized laticifer cells and profilins from other tissues of the plant.

We expressed the *H. brasiliensis* latex profilin as a soluble nonfusion protein in *E. coli* and applied the recombinant protein in immunoblot and ELISA experiments to study the occurrence of Hev b 8-specific IgE antibodies in

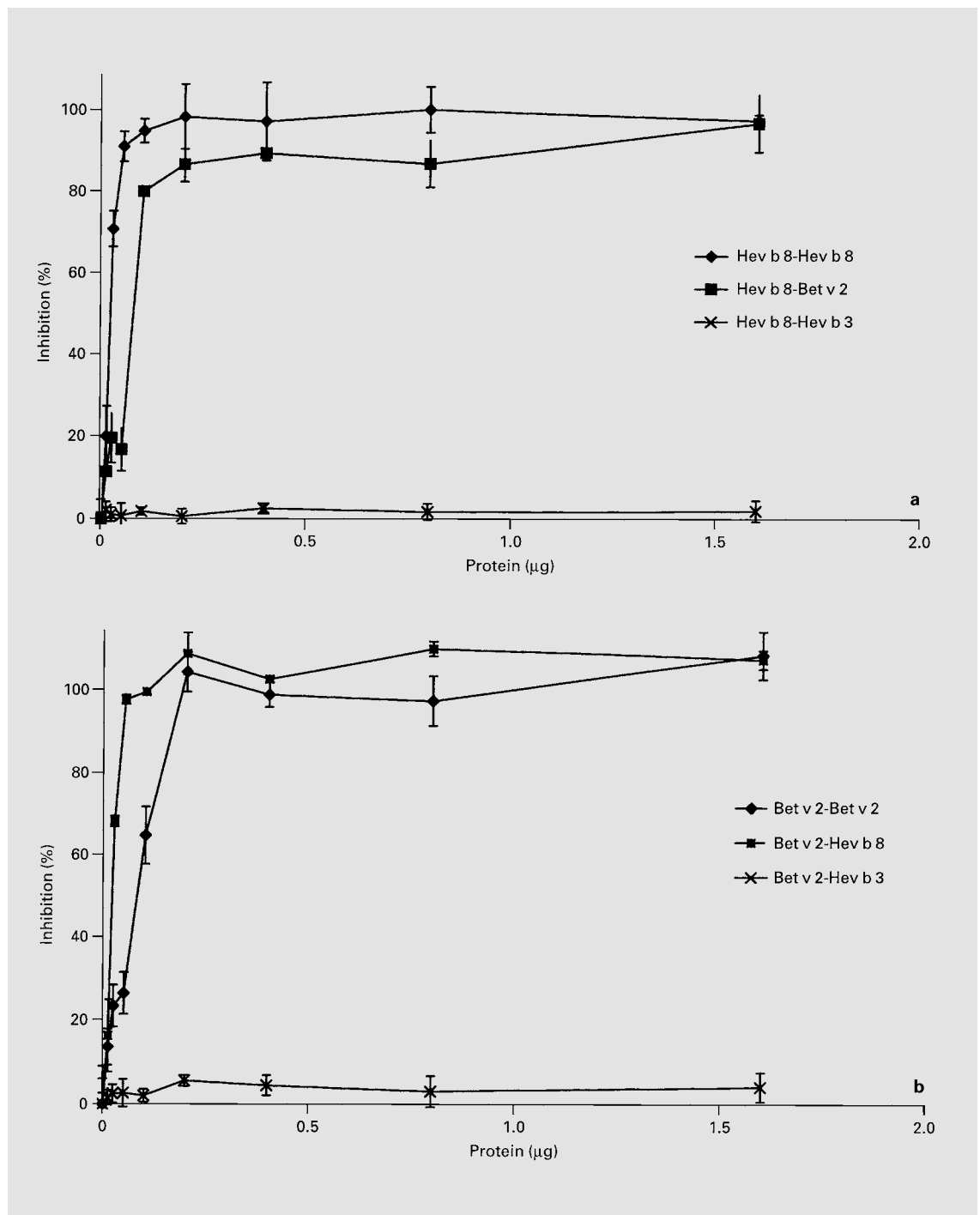


Fig. 4. ELISA inhibition with rHev b 8 and rBet v 2. Microtiter plates were coated with 4 μg/ml rHev b 8 (**a**) and 4 μg/ml rBet v 2 (**b**). A pool of allergic patients' sera containing rHev b 8-specific IgE was preincubated with different concentrations of rHev b 8, rBet v 2 or rHev b 3 as a negative control. Bound IgE was detected with an alkaline phosphatase-conjugated anti-IgE antibody. Samples were prepared in duplicate. Vertical bars indicate standard deviations.

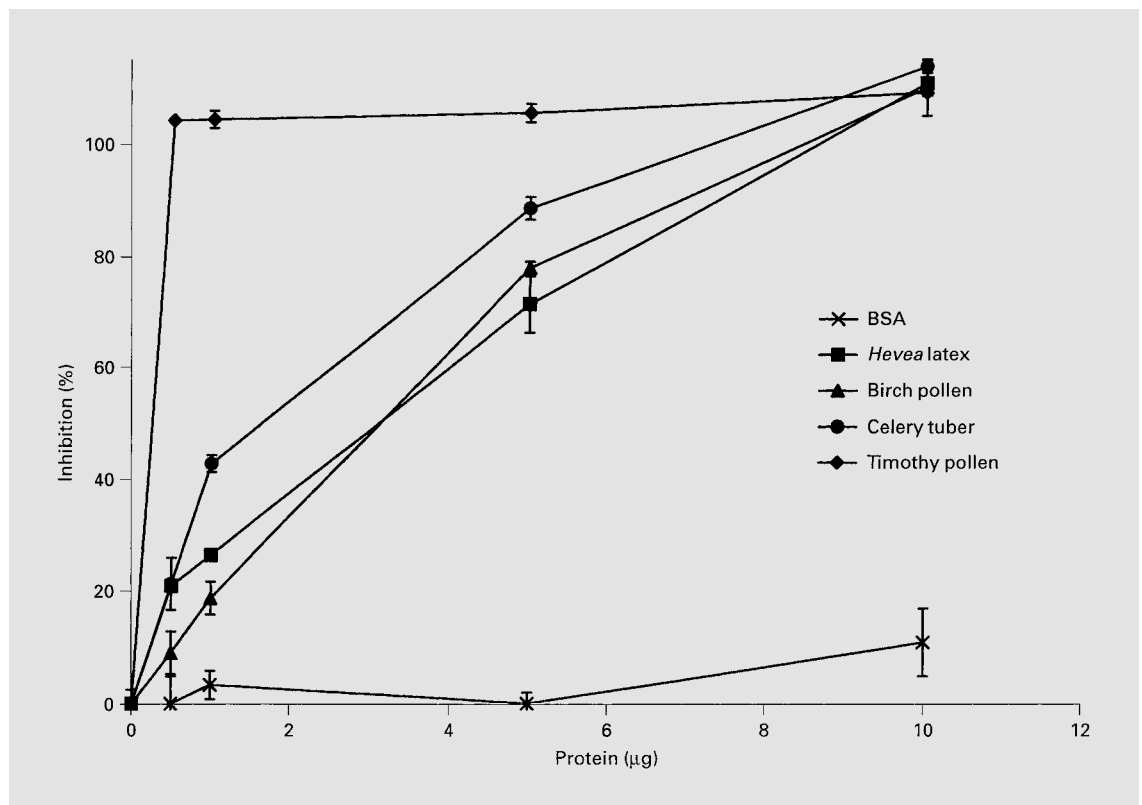


Fig. 5. ELISA inhibition with natural plant protein extracts. Microtiter plates were coated with 4 µg/ml rHev b 8. A pool of allergic patients' sera containing rHev b 8-specific IgE was preincubated with different concentrations of protein extracts from *Hevea* latex, birch pollen, celery tuber and timothy pollen. For control purposes, preincubation with BSA was carried out. Samples were prepared in duplicate. Vertical bars indicate standard deviations.

well-defined latex-allergic patient groups. The prevalence of sensitization to *Hevea* latex profilin among the latex-allergic HCWs was 24% and in the SB group 6%. The frequency of IgE to rHev b 8 in the group of latex-allergic HCWs with food and/or pollen allergy was 32%. In contrast, latex-allergic HCWs without an associated pollen or food allergy did not show rHev b 8-specific IgE. Likewise, the two SB patients with IgE to rHev b 8 had either a food or a pollen allergy. It is now tempting to speculate about the primary sensitizing agent to Hev b 8. From these data, we conclude that primary sensitization to profilin takes place via pollen or food profilins in the majority of cases. The route of primary sensitization to Hev b 8 by latex products seems to be of lesser importance. This concurs with the finding that the profilin concentration in latex products is very low [25].

The observed frequent in vitro reactivity to rHev b 8 does not necessarily imply the clinical relevance of Hev b 8-specific IgE in all patients. In a paper by Yip et al.

[38], it was shown that only a small fraction of patients with a positive RAST to nHev b 8 showed a concomitant positive skin prick reaction. Therefore, in vivo studies using rHev b 8 will be necessary to elucidate the clinical relevance of this allergen.

We were able to show that nHev b 8 and rHev b 8 exhibited in vitro cross-reactivities with profilins from birch pollen, timothy pollen and celery tuber. rHev b 8 was able to abolish IgE binding to profilins present in protein extracts from *Hevea* latex, birch pollen, timothy pollen and celery tuber (fig. 3). Moreover, profilin-specific IgE was inhibited by rHev b 8 and rBet v 2 in a comparable manner in ELISA experiments (fig. 4a, b). In turn, natural profilins present in extracts from *Hevea* latex, birch pollen, timothy pollen and celery tuber were able to inhibit profilin-specific IgE binding to rHev b 8 (fig. 5).

The high sequence similarities and equivalent immunological characteristics of plant profilins justified the modeling of the structure of Hev b 8 based on the experi-

mentally determined crystal structures of Bet v 2 [28] and the *A. thaliana* profilin [29]. This homology model was then used to localize the linear IgE epitopes of Bet v 2 on the structure of Hev b 8. All three epitopes are found on the accessible surface of the molecule. Only the N-terminal IgE epitope is located in a region with variable structure, but the orientation of the N-terminal α -helix most likely will not influence the binding of IgE to a linear epitope. The adjoining loop cannot be reliably modeled because it comprises a highly flexible region whose experimentally determined crystal structure will not reflect the situation in solution. Potential cross-reactive conformational epitopes may be located almost everywhere on the surface due to the virtually identical structures of different plant profilins. The high sequence identity between Hev b 8 and the template proteins (82% to birch profilin, 72% to the profilin from *Arabidopsis*) implies that the model structure is accurate enough for epitope prediction. In the course of the 3DCrunch project [27], more than 1,200 models of proteins with known three-dimensional structures have been generated. Eighty-two percent of the models based on templates with 70–79% sequence identity to the query sequence showed a significant correspondence with the experimental structures, defined by a root mean square deviation of less than 3 Å (see http://www.expasy.ch/swissmod/SM_LikelyPrecision.html).

In a recently published study by Rihs et al. [26], the immunological properties of a recombinant *H. brasiliensis*

profilin whose cDNA had been amplified from leaf RNA were examined. It was observed that 2 out of 5 Hev b 8-sensitized patients showed no IgE binding to rBet v 2. From this, the authors concluded the presence of non-cross-reactive IgE epitopes on Hev b 8. This is in conflict with our findings that profilin-specific IgE from all Hev b 8-sensitized patients bound to the profilin from birch pollen. This discrepancy can be explained by the fact that Rihs et al. [26] performed their experiments with a recombinant profilin derived from RNA from *Hevea* leaves, whose sequence is quite different from *Hevea* latex profilins and has a low sequence identity (75%) with Bet v 2. In our experiments using sera containing rHev b 8-specific IgE, the cross-reactivity between the profilins from latex, pollen and food was obvious. From these data, we conclude that pollinosis and food-allergic patients with profilin-specific IgE can be at risk of developing a latex allergy.

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