

Original article

PCR-based cloning, isolation, and IgE-binding properties of recombinant latex profilin (rHev b 8)

Background: Profilin (Hev b 8) in natural rubber latex (NRL) has been assumed to be an important allergen. Since latex profilin has a molecular mass similar to two other latex allergens (Hev b 1 and Hev b 6.03) in the 14-kDa range, it is difficult to obtain sufficient amounts of purified native profilin for investigations and diagnostics. The present study aimed to produce recombinant latex profilin (rHev b 8) and study its IgE-binding reactivity.

Methods: A profilin-specific cDNA encoding the latex profilin from *Hevea brasiliensis* leaves was synthesized and subcloned, and the rHev b 8 was overexpressed in fusion with the maltose-binding protein (MBP) in *E. coli*. The IgE-binding reactivity of rHev b 8 was studied by immunoblotting, immunoblot inhibition experiments, and the Pharmacia CAP method, with 25 sera from health-care workers with latex allergy and 17 sera from latex-sensitive spina bifida patients.

Results: rHev b 8 was found to have 131 amino acids and a sequence identity of 75% with birch profilin (Bet v 2). Analysis by the CAP system revealed the presence of rHev b 8-specific IgE antibodies in two out of 17 sera from spina bifida patients and in five out of 25 sera (20%) from health-care workers. Two subjects of the latter group with rHev b 8-specific IgE showed negative results in the skin prick tests with tree-pollen extracts and had no IgE to rBet v 2, indicating the presence of IgE-binding epitopes on the Hev b 8-molecule which do not cross-react with birch profilin. Immunoblot inhibition assays using MBP-rHev b 8 as inhibitor confirmed the presence of latex profilin in the NRL extract. IgE binding to the native latex profilin could be completely inhibited by the MBP-rHev b 8.

Conclusions: Latex profilin represents a minor allergen in NRL and may have IgE-binding epitopes different from Bet v 2.

H.-P. Rihs, Z. Chen, P. Rozynek, X. Baur

Research Institute for Occupational Medicine (BGFA) at the Ruhr-University, Bochum, Germany

M. Lundberg

MIAB, Uppsala, Sweden

R. Cremer

Children's Hospital Cologne, Cologne, Germany

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Hans-Peter Rihs, PhD
Berufsgenossenschaftliches Forschungsinstitut für
Arbeitsmedizin (BGFA)
Abteilung Molekulare Genetik
Buerkle-de-la-Camp-Platz 1
44789 Bochum
Germany

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Natural rubber latex (NRL) is widely used in medical devices as well as in certain everyday articles (reviewed in ref. 1). So far, 10 different latex allergens have been included in the latest IUIS nomenclature list (2). By cross-inhibition experiments using profilins purified from different plants, Vallier et al. (3) found that the latex profilin (Hev b 8) has an IgE-binding potential but is a minor allergen in NRL: only two out of 19 latex-allergic subjects were found to have specific IgE antibodies to latex profilin. Nieto et al. (4) recently reported a much higher prevalence of IgE antibodies to latex profilin in patients with latex allergy. They found that all 24 spina bifida (SB) patients with latex allergy tested had positive skin responses to Hev b 8 in skin prick tests (SPT), whereas atopics with fruit allergy did not show a reaction to the profilin. SPT with latex profilin in a group of 17 adult latex-allergic subjects also showed a high reaction frequency (95%). Specific IgE antibodies to latex profilin were observed in 14 out of 24 sera (58%) tested.

Our efforts to isolate the latex profilin revealed that this protein is present in NRL at a very low concentration. Furthermore, the latex allergen rubber elongation factor (Hev b 1), which was identified as a major allergen especially in children with SB and latex allergy (5), and the C-domain of prohevein (Hev b 6.03) (6) have a molecular mass similar to the latex profilin in the 14-kDa range. Since the separation of proteins with the same molecular mass in NRL is difficult and time-consuming, we decided to produce latex profilin by recombinant methods.

Recently, we designed highly degenerate primers allowing the identification of the wheat profilin sequence (7), as well as the soybean profilin sequence (8), by PCR-based cDNA cloning. In the present study, this approach was used to identify latex profilin from *Hevea brasiliensis* leaves (EMBL accession no. AJ132397) in order to decode the primary sequence of latex profilin and to elucidate its IgE-binding properties with sera from latex-allergic individuals.

Table 1. Clinical data: total and specific IgE values of 25 latex-allergic health-care workers (HCWs) tested by CAP method

Subject no.	Age (years)/sex	Total IgE (kU/l)	Latex-associated symptoms	Latex-specific IgE (kU/l)	MBP-rHev b 8-specific IgE (kU/l)	rBet v 2-specific IgE (kU/l)
1*	27/F	831	A, C, R, U	10.20	1.07	<0.35
3	29/F	170	C, R, U	8.41	4.58	4.33
7*	29/F	23.9	C, R, U	4.38	1.01	<0.35
19	43/F	>2000	A, C, R, U	5.31	8.56	7.27
25	28/F	279	A, R, U	6.21	16.10	14.60
2	31/F	717	A, R, U	3.66	<0.35	Nd
4*	34/F	603	C, R, U	11.90	<0.35	Nd
5*	44/F	Nd	C, R, U	3.23	<0.35	Nd
6*	52/F	219	C, R, U	15.70	<0.35	Nd
8*	34/F	104	A, R, U	6.53	<0.35	Nd
9*	33/F	111	A, R, U	4.64	<0.35	Nd
10*	39/F	523	R, U	7.34	<0.35	Nd
11*	46/F	31.1	R, U	4.55	<0.35	Nd
12*	43/F	157	C, R, U	25.00	<0.35	Nd
13*	23/F	41.3	A, R, U	3.13	<0.35	Nd
14*	45/F	207	C, R, U	11.70	<0.35	Nd
15*	32/F	>2000	A, C, R, U	1.39	<0.35	Nd
16	49/M	108	A, C, R, U	2.71	<0.35	Nd
17	29/F	591	A, C, R, U	7.52	<0.35	Nd
18*	35/F	79.5	C, R, U	5.70	<0.35	Nd
20	32/F	193	A, C, R, U	1.30	<0.35	<0.35
21*	44/F	135	A, R, U	5.77	<0.35	<0.35
22*	33/F	80.3	A, C, R, U	7.82	<0.35	Nd
23*	32/M	29.0	A, C, R, U	2.79	<0.35	Nd
24	21/F	500	A, C, R	1.63	<0.35	Nd

* Skin prick test negative to tree-pollen mixtures.

A: asthma; C: conjunctivitis; Nd: not determined; R: rhinitis; U: urticaria.

Material and methods

Human sera

Serum samples were obtained from 25 latex-allergic health-care workers (HCWs) (23 women and two men, aged 21–52 years, mean age: 35.5 years), who showed positive skin reactions to latex extracts in SPT and the presence of IgE antibodies to NRL proteins as tested by the Pharmacia CAP system (Pharmacia-Upjohn, Uppsala, Sweden). All HCWs also had latex-associated symptoms (summarized in Table 1). The concentrations of specific IgE antibodies to NRL were found to be 1.30–25 kU/l. We also tested sera from 17 latex-sensitive SB patients (five girls, 12 boys, aged 2–21 years, mean age: 11.4 years). Eleven out of these patients showed a positive SX1 test, a screening test for environmental allergens; 10/17 patients (59%) had latex-associated symptoms. Six sera from subjects without latex allergy were used as controls.

SPT

SPT were performed in all HCWs with common environmental aeroallergens including tree- and grass-pollen mixtures (HAL, Düsseldorf, Germany) and with latex extract prepared in our laboratory (total protein concentrations: 1, 10, and 100 µg/ml in saline containing 0.4% phenol). Cutaneous reactions were read after

Table 2. Clinical data: total and specific IgE values of 17 latex-sensitized spina bifida (SB) patients tested by CAP method

Subject no.	Age (years)/sex	Total IgE (kU/l)	Latex-associated symptoms (operations)	Latex-specific IgE (kU/l)	MBP-rHev b 8-specific IgE (kU/l)	rBet v 2-specific IgE (kU/l)
SB1*	21/M	258	None (10)	2.82	0.92	1.21
SB2*	10/M	784	U (7)	>100	18.30	13.70
SB3*	9/M	>2000	An, B, I (9)	>100	<0.35	<0.35
SB4*	17/M	170	An, I, U (24)	25.50	<0.35	<0.35
SB5*	14/F	169	None (4)	4.06	<0.35	<0.35
SB6*	18/F	272	O (8)	18.60	<0.35	<0.35
SB7*	14/F	397	O, U (4)	79.80	<0.35	<0.35
SB8*	13/M	829	O, U (15)	>100	<0.35	<0.35
SB9*	7/F	288	B, C, E, R, O, U (9)	53.90	<0.35	<0.35
SB10*	19/M	227	None (5)	1.34	<0.35	<0.35
SB11*	8/M	60.7	C, R (9)	8.20	<0.35	<0.35
SB12	2/F	104	None (2)	1.40	<0.35	<0.35
SB13	4/M	105	None (8)	12.30	<0.35	<0.35
SB14	8/M	96.1	O (8)	20.60	<0.35	<0.35
SB15	17/F	35.3	I, O, U (6)	1.02	<0.35	<0.35
SB16	5/M	188	None (4)	8.14	<0.35	<0.35
SB17	6/M	576	None (7)	20.01	<0.35	<0.35

* Positive in screening test for environmental allergens (SX1-test, CAP system). An: anaphylaxis; B: bronchospasm; C: conjunctivitis; E: erythema; O: edema; R: rhinitis; U: urticaria.

15 min and considered positive when a wheal reaction was at least 2 mm and surrounded by erythema, as compared with negative control values (wheal <2 mm, no erythema). In the case of SB patients, SPT with latex extract were performed in only three cases (SB no. 1, SB no. 5, and SB no. 9 in Table 2). Two patients (SB no. 5 and SB no. 9) showed a positive, one patient (SB no. 1) a negative result.

Polymerase chain reaction (PCR) of the cDNA

cDNA was obtained from total RNA of *H. brasiliensis* leaves, as previously described (9). The latex profilin-specific amplification was carried out in a 50-µl reaction mixture containing 1 ng latex cDNA, 50 mM KCl, 10 mM Tris-HCl, pH 9.0, 2.0 mM MgCl₂, 0.01% (w/v) gelatin (Sigma, Deisenhofen, Germany), 500 µM of deoxynucleotide triphosphate (dNTP), 25 pmol of each of the primers PRO52 (5'-ATGTCNTGGCAR-GCNTAYGT-3') and PRO33 (5'-ARTADCCYTGN-TCDATNAGRTART-3') (7, 8), and 2.5 units Taq Polymerase (Life Technologies, Karlsruhe, Germany). Reaction products were obtained in a thermal cycler (Biometra, Göttingen, Germany) with an initial denaturation step (3 min for 95°C) and a total of 35 cycles of denaturation (95°C for 1 min), annealing (57°C for 1 min), and extension (72°C for 2 min followed by 10 min at 72°C).

Subcloning and sequencing of PCR products

The 394 bp-PCR product was first subcloned into the vector pCR2.1 with the TA cloning kit (Invitrogen,

Leek, The Netherlands). Clones carrying inserts were characterized by restriction analysis and by non-radioactive sequencing on an ABI 310 sequencer (Applied Biosystems, Weiterstadt, Germany). Sequencing analysis revealed that the PCR products correspond to a 394-bp open reading frame named La PRO 2.

For the efficient expression of the recombinant latex profilin in *E. coli*, TB-1, the complete LaPRO 2 sequence, was transmitted after primer-mediated adaptation into the expression vector pMAL-c2 (New England Biolabs, Schwalbach, Germany) downstream from the *malE* gene coding for the maltose-binding protein (MBP). An amount of 500 ng LaPRO 2-DNA was used as target for the amplification with primer pair Hev b 8-5' (5'-GGGACACATGTCTGGCAGGCGTACGTAG-3') and Hev b 8-3' (5'-GCGAAGCTTARTAGCCCTGRTCRATSAGGTAGT-3') under the same PCR conditions as described above, with the exception that the annealing temperature was 60°C, and 40 cycles were run. After a *PshA I-Hind III* digest, the insert was subcloned into *XmnI-EcoRI*-digested pMAL-c2, yielding pMALrHev b 8, which could overexpress a 56.8-kDa MBP-latex profilin fusion protein in the presence of 0.3 mM IPTG in the *E. coli* host TB-1 (New England Biolabs, Schwalbach, Germany).

Expression and purification of MBP-rHev b 8

A volume of 1 l Luria broth (LB) medium containing 100 µg/ml (w/v) ampicillin and 2 mg/ml (w/v) glucose was inoculated with 10 ml fresh recombinant *E. coli* cells from an overnight culture and incubated at 37°C for 2 h on a laboratory shaker (New Brunswick, Nuertingen, Germany). Expression of the fusion protein was induced by shaking in the presence of 0.3 mM IPTG (Roth, Freiburg, Germany) for 4 h at 37°C. *E. coli* cell lysates containing the expressed fusion protein were prepared by sonification in an ice-water bath with a Branson sonifier-250 (eight intervals of 20 s on level 4, followed by a time stop of 30 s), followed by centrifugation at 10 000 g for 30 min. The supernatant was loaded onto a 1.5 × 20.0 cm column filled with a 7 ml bed volume of amylose (New England Biolabs, Schwalbach, Germany). Equilibration, washing, and elution were performed as recommended by the supplier's protocol (New England Biolabs, Schwalbach, Germany).

The rHev b 8 could be cleaved from MBP by incubating affinity-purified fusion protein with proteinase factor Xa (MobiTech, Goettingen, Germany), in which the lyophilized factor Xa was dissolved in 100 mM phosphate-buffered saline adjusted to pH 7.6 and used at a factor Xa:fusion protein ratio of 1:20 (w/w) for 8 h at room temperature under smooth shaking on a vortexer in the presence of 0.08% (v/v) SDS.

Determination of specific IgE antibodies by the CAP method

For the determination of rHev b 8-specific IgE, MBP-

rHev b 8 fusion protein was immobilized onto a cellulose solid phase by covalent binding. The coupling concentration was adjusted to attain a linear measurement range and a background below 0.35 kU of allergen-specific IgE per liter (kU/l). The capture antigen (ImmunoCAP of rHev b 8) was then used to determine IgE antibodies to rHev b 8 by Pharmacia CAP. A specific IgE value of >0.35 kU/l was regarded as positive. Total IgE, latex-specific IgE, and rBet v 2-specific IgE were also determined by the CAP system, as recommended by the supplier.

Electrophoresis and immunoblotting

One-dimensional SDS-PAGE and immunoblot analysis of the fusion protein, as well as the factor Xa-cleavage products, were performed with 100 µg rHev b 8 on a

LaPRO 2:	1	ATGTCGTGGCAAGCGTATGTAGATGATCATCTTATGTGCGAAATCGAGGG	50
Y15042.1	1	M S W Q A Y V D D H L M C E I E G	
Bet v 2		: : : : T : : : E R : : : : : : : :	
LaPRO 2:	51	CAATCATCTCTCTGCTGCCGCCATCATCGGCCAAGACGGCAGCGTTTGGG	100
Y15042.1	18	N H L S A A A I I G Q D G S V W	
Bet v 2		: : : T : : : : : : : : : : : :	
LaPRO 2:	101	CCGAGAGCGCCAACTTCCCTCAGTTCAAGTCTGAAGAAATCACTGGCATT	150
Y15042.1	34	A Q S A N F P Q F K S E E I T G I	
Bet v 2		: : : S : : : : : : : P Q : : : : :	
LaPRO 2:	151	ATGAGTGATTTTACGAACTGGAACGCTGCTCCAAGTGGATTGTACAT	200
Y15042.1	51	M S D F H E P G T L A P T G L Y I	
Bet v 2		: : : D : : : : : : : : : : : H L	
LaPRO 2:	201	TGGTGGCACAAATACATGGTGATCCAAAGCGAGCCAGGAGCTGTCATT	250
Y15042.1	68	G G T K Y M V I Q G E P G A V I	
Bet v 2		: : : : : : : : : : : A : : : :	
LaPRO 2:	251	GGGGGAAGAAGGGCTCTGGTGGTGTACTGTGAAGAAGACTAATCAGGCC	300
Y15042.1	84	R G K K G P G G V T V K K T N Q A	
Bet v 2		: : : : : : : : : : : R : : : : :	
LaPRO 2:	301	TTAATAATTGGTATCTACGATGAGCCAATGACTCCAGGGCAGTGCAACAT	350
Y15042.1	101	L I I G I Y D E P M T P G Q C N M	
Bet v 2		: : : : : : : : : : : V F : : : : :	
LaPRO 2:	351	GATGTGTGAAGGCTTGGCGACTACCTCATTGATCAGGGCTATTAA	396
Y15042.1	118	I V E R L G D Y L I D Q G Y STOP	
Bet v 2		V : : : : : : : : : : : L STOP	

Figure 1. Nucleotide sequences and deduced amino-acid sequences of latex profilin gene (LaPRO2) from *H. brasiliensis* cDNA. Numeric order starts with open reading frame of latex-profilin cDNA. Coding regions are denoted by capital letters up to last nucleotide of primer PRO33 (T in position 394). Letters in italics (AA) stem from 3' primer Hev b 8-3' described in Methods section. Amino-acid residues are shown in one-letter code. Only amino-acid residue substitutions of latex-pollen profilin (Y15042.1) and Bet v 2 (M65179.1) are shown. Colon indicates that amino-acid residue is identical with that shown for LaPRO2. Underlined amino-acid residues show two additional residues present only in Bet v 2.

10% NuPAGE Bis-Tris gel (NOVEX, San Diego, CA, USA) under reducing conditions, as described recently (10). In the case of latex C-serum, 300 µg/gel was used.

Immunoblot inhibition of IgE binding to profilin in NRL

For immunoblot inhibition experiments, 100 µl of a single serum showing IgE antibodies to NRL, as well as to the MBP-rHev b 8, was diluted 1:10 and preincubated with 50 µg MBP-rHev b 8 by shaking for 3 h at room temperature. The serum samples were then reacted with latex C-serum-blotted membrane strips. The presence of natural profilin in the latex C-serum was detected by comparing the immunoblotting results with those obtained by the immunoblot inhibition assays.

Results

Amplification of latex profilin cDNA by PCR and sequence analysis

The PCR amplification of latex cDNA with degenerate primers produced a single 394-bp fragment. The cDNA (LaPRO2) was obtained twice by this procedure. The nucleotide sequence and the corresponding deduced amino-acid sequence of LaPRO2 (Fig. 1) carried an open reading frame coding for 131 amino-acid residues with a predicted average molecular mass of 14.15 kDa and a theoretic isoelectric point value of 4.6. The highest amino-acid identity (90%) was observed between the sequence of LaPRO2 and an amino-acid sequence describing another latex profilin recently isolated from *Hevea* pollen (EMBL accession no. Y15042.1, Fig. 1). The comparison with the sequences of the plant profilins

of *Phaseolus vulgaris* (89%; accession no. X81982) (11), *Glycine max* (87%, accession no. O56809) (8), *Arachis hypogea* (83%, accession no. AF059616) (12), and *Cynodon dactylon* (Bermuda grass) (81%, accession no. O04725) (13) also revealed higher sequence identity. A sequence identity of 75% was found when compared with the well-characterized birch profilin Bet v 2 (accession no. M65179.1, Fig. 1) (14).

Expression, purification, and analysis of latex profilin rHev b 8

For the efficient expression of rHev b 8, the insert cDNA fragment LaPRO2 coding for the full-length latex profilin was subcloned into the pMAL vector system. The expression by IPTG induction generated a fusion protein, MBP-rHev b 8, with an estimated molecular mass of 56.8 kDa (Fig. 2, lane 1) corresponding to the mass of 14.1 kDa for the rHev b 8 plus the mass of 42.7 kDa for the MBP. The yield of affinity-purified recombinant MBP-rHev b 8 fusion protein was about 4 mg/l (mean of four preparations) according to the analysis by the Bradford method. The isolated fusion protein had a purity of more than 95% as shown by SDS-PAGE (Fig. 2, lane 2).

Factor Xa cleavage of rHev b 8

rHev b 8 can be cleaved from the MBP carrier protein by factor Xa digest. Best results were obtained in the presence of 0.08% SDS and a cleavage time of 8 h, yielding an almost complete cleavage of the 42.7-kDa MBP carrier protein from the 14.1-kDa target protein rHev b 8 (Fig. 2, lane 3).

Immunologic characterization of rHev b 8

Experiments using the CAP method were performed to study the IgE-binding reactivity of rHev b 8 and the prevalence of IgE to Hev b 8 among the latex-allergic patients. ImmunoCAPs coupled with MBP-rHev b 8 were used as the capture antigen, and 25 sera from patients with latex allergy, as well as 17 sera from latex-sensitive SB patients and showing the presence of IgE antibodies to latex, were tested. As shown in Table 1, five out of the 25 (20%) sera had specific IgE antibodies to rHev b 8 (1.01–16.1 kU/l). In the 17 latex-sensitive SB patients tested (Table 2), two subjects showed IgE antibodies to rHev b 8 (0.92–18.3 kU/l). These seven sera were also tested for IgE to birch-pollen profilin (rBet v 2), and two HCWs (nos. 1 and 7 in Table 1) were found to have no rBet v 2-specific IgE antibodies. These two subjects also showed hevein-specific IgE antibodies when tested by the enzyme allergosorbent test (EAST) (data not shown). SPT results revealed that they also had negative skin-prick reactions when tested with tree-pollen mixtures. These results demonstrate the presence of rHev b 8-specific IgE antibodies that do not cross-react with birch-pollen profilin.

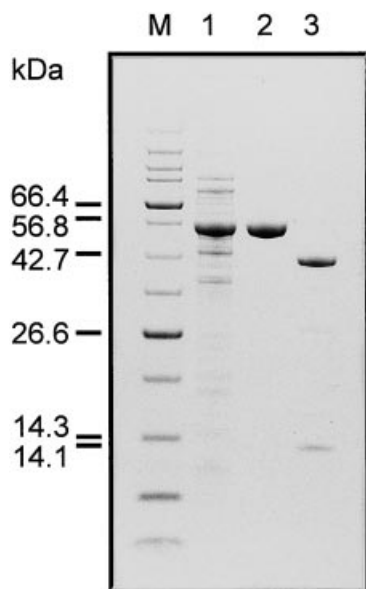


Figure 2. Expression, purification, and factor Xa digestion of MBP-rHev b 8 on 10% NuPAGE Bis-Tris gel. From left to right, molecular mass marker (M), aliquot of crude extract after IPTG induction (lane 1), and 5 µg affinity-purified MBP-rHev b 8 before (lane 2) and after factor Xa digest (lane 3) are shown.

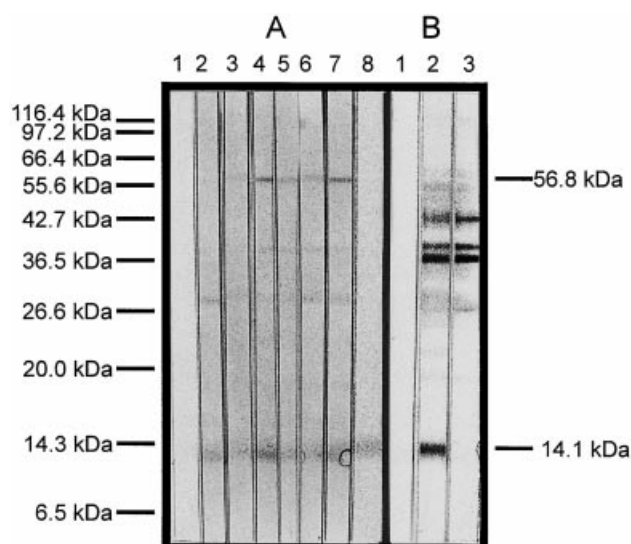


Figure 3. Results of immunoblot (A) and immunoblot inhibition (B) experiments: digestion mixture of MBP-rHev b 8 with enzyme factor Xa was used in immunoblotting and IgE binding. Almost completely cleaved rHev b 8 (14.1 kDa) was detectable in five sera of latex-allergic HCWs (A, lanes 2–6, nos. 1, 3, 7, 19, and 25) and two sera of latex-sensitive SB patients (A, lanes 7 and 8, SB1, SB2) showing rHev b 8-specific IgE antibodies tested by CAP method. Negative serum pool (A, lane 1) showed no reaction. In immunoblot inhibition experiments, latex-C serum proteins were separated and blotted (Fig. 3B). One serum was tested in absence (B, lane 2) and in presence (B, lane 3) of inhibitor MBP-rHev b 8. Latex-negative serum pool showed no reaction (B, lane 1).

Specific IgE antibodies binding to rHev b 8 could also be demonstrated by immunoblot analysis. A digestion mixture of MBP-rHev b 8 with the enzyme factor Xa was separated by SDS-PAGE and blotted onto a PVDF membrane. Immunostaining results (Fig. 3A) showed that all sera from subjects with rHev b 8-specific IgE antibodies tested by the CAP method recognized the rHev b 8 cleaved from MBP-fusion protein (Fig. 3A, lanes 2–8), whereas no IgE binding was observed to MBP alone (42.7 kDa). A pooled serum sample comprising six sera of subjects without latex allergy served as negative control without IgE-binding reactivity (Fig. 3A, lane 1).

Fig. 3B demonstrates the presence of latex profilin in latex C-serum after immunoblot inhibition of the IgE binding to native latex profilin with MBP-rHev b 8-recombinant latex profilin as inhibitor. In this experiment, an aliquot of latex C-serum extract was run on a 10% BisTris gel and blotted onto a PVDF membrane. Membrane strips were then incubated with a single serum diluted 1:10 (Fig. 3B, lanes 2 and 3) in the absence (Fig. 3B, lane 2) and in the presence (Fig. 3B, lane 3) of MBP-rHev b 8 (50 µg/ml). As shown in Fig. 3B, lane 3, the IgE-binding to a protein in the 14-kDa area could be completely inhibited by pre-incubation with MBP-rHev b 8, whereas the other IgE-binding bands were not affected, indicating that latex profilin is present in NRL extract and has the same

IgE-binding reactivity as rHev b 8. The serum used in this experiment also showed positive IgE-binding to hevein when tested by EAST (data not shown).

Discussion

Natural sources of rubber latex can be of varying composition (15) and are difficult to standardize (16). The preparative isolation of certain allergens from these sources is time-consuming and thus expensive. If no post-translational modifications occur, the cloning production and isolation of recombinant allergens in *E. coli* is an economical alternative and may provide sufficient amounts of highly purified proteins for diagnostics and therapeutics, as well as for experimental purposes.

In the present study, we have chosen the latter approach to identify the latex profilin-coding sequence of *H. brasiliensis* leaves by cDNA cloning using RT-PCR techniques and highly degenerated primers. The recombinant latex profilin has 131 amino-acid residues with a calculated pI of 4.6 and a molecular mass of 14.15 kDa. To our knowledge, this is the first successful expression and purification of a recombinant latex profilin. In 1997, Asturias et al. had already presented a coding sequence for a *H. brasiliensis* pollen profilin from the cultivar RRIC133 in the gene bank with the accession number Y15042.1. The deduced amino-acid sequence of this clone showed the highest homology (90%) to our recombinant latex profilin rHev b 8 (Fig. 1). Sequence differences in both latex profilins may be due to the fact that we used the cultivar RRIM600 of *H. brasiliensis*.

The allergenicity of the rHev b 8 was studied by testing its IgE-binding reactivity by the well-established CAP method. The recombinant Hev b 8 fusion protein was used for the immobilization of the solid phase, and 25 sera from latex-allergic HCWs (Table 1), as well as 17 sera of latex-sensitive SB patients (Table 2), were tested. Five HCW sera (20%) and two SB sera (12%) revealed rHev b 8-specific IgE antibodies. In the immunoblot experiments, all sera showing IgE to rHev b 8 in the CAP system also recognized the rHev b 8 free of the MBP carrier protein; no IgE-binding reactivity was observed to MBP alone. The fact that IgE antibodies do not bind to MBP could also be confirmed by EAST using MBP-coated disks as the solid phase (data not shown). Immunoblot inhibition experiments performed with MBP-rHev b 8 fusion protein as inhibitor (Fig. 3B) showed that the latex profilin is present in the latex C-serum fraction and that it is able to bind to IgE antibodies.

Recent articles about the role of latex profilin in latex-sensitized subjects show conflicting results. IgE inhibition experiments with purified ragweed profilin as inhibitor revealed that latex profilin is mainly present in the C-serum, and that smaller amounts can also be

found in the rubber fraction (3). In this study, two out of 19 tested latex-sensitive subjects (11%) had antiprolin IgE antibodies. In contrast, Nieto et al. (4) purified profilins with three different molecular masses (10.2, 14.2, and 15.7 kDa) from liquid latex, and reported 100% positive SPT reactions in 24 latex-allergic spina bifida patients and specific IgE to latex profilin in 14 out of 24 sera (58%). Furthermore, they investigated 17 latex-allergic adults and found that 95% of them had positive skin prick tests, whereas only six of them (35%) had positive latex-profilin-specific IgE.

The IgE prevalence obtained in our study on the latex-allergic HCW group (Table 1) is in good agreement with the results of Vallier et al. (3) as well as with those reported for profilins from other plant species such as birch-pollen (17, 18) and olive pollen (19), showing frequencies of 20% and 24%, respectively, in birch and olive pollen-allergic patients. Regarding the IgE-binding reactivity in latex-sensitive spina bifida patients, we found specific IgE to recombinant latex profilin in only

two out of 17 sera (12%) tested. Interestingly, two out of the five latex-allergic HCWs with IgE to rHev b 8 showed negative skin prick reactions when tested with tree-pollen mixtures including birch and hazel, and had no IgE antibodies to birch-pollen profilin rBet v 2. These findings indicate that latex-allergic patients may be sensitized directly by contact with profilin in NRL. However, a cross-reaction caused by presensitization to other plant profilins cannot be excluded, and this possibility needs further investigation. From our present data, we conclude that latex profilin is a minor allergen in NRL.

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