

cDNA cloning of the 43-kDa latex allergen Hev b 7 with sequence similarity to patatins and its expression in the yeast *Pichia pastoris*

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IgE-mediated hypersensitivity to latex proteins present in health care products, particularly in latex gloves, has become an important public health problem in recent years. We purified natural Hev b 7, a 43-kDa patatin-like allergen from the latex of *Hevea brasiliensis* and determined several internal peptide sequences. A heterologous hybridization probe of a patatin gene of potato, to which these peptides could be aligned best, was used to screen a latex cDNA library. The cDNA encoded an acidic protein of 388 amino acids with a molecular mass of 42.9 kDa. The deduced amino acid sequence had 39–42% identity to patatins from *Solanum tuberosum*. The purified recombinant Hev b 7 expressed in the yeast *Pichia pastoris* displayed, similarly to patatins from *S. tuberosum*, esterase activity. Both natural and recombinant Hev b 7 were recognized by IgE from sera of latex-sensitized allergic individuals. In contrast to patatins from *S. tuberosum* and *Nicotiana tabacum*, natural Hev b 7 lacked an N-terminal leader peptide for targeting to the endoplasmatic reticulum and was not glycosylated. These results establish the 43-kDa patatin-like protein as a latex allergen and raise the possibility of different cellular localization and function compared to *S. tuberosum* patatins.

Keywords: latex allergy; Hev b 7; *Hevea*; *Pichia pastoris*; type-I allergy.

IgE-mediated hypersensitivity to natural rubber latex (NRL) has become a serious medical problem especially among health care workers and patients who have to undergo repeated surgeries such as patients with spina bifida [1, 2]. The increase in allergic reactions to latex has been partially attributed to the enormous increase in the use of latex gloves for protection against HIV and hepatitis B and C [3, 4].

In fresh NRL, the source material for manufactured latex products, a variety of IgE-binding proteins with molecular masses ranging over 5–110 kDa were detected by two-dimensional immunoblotting [5–7]. In 1993, the first latex allergen was identified at the molecular level as the rubber elongation factor [8]. Since then, a total of eight NRL allergens have received an international nomenclature designation [9]. The full coding sequence is only known for Hev b 1, Hev b 2 (a β -1,3-glucanase), Hev b 5 (an acidic protein with similarity to a protein found in kiwi fruit), Hev b 6 (prohevein) and Hev b 8 (profilin), all reviewed in [9]. Detailed information about molecular features of the most significant latex allergens is a prerequisite for manufacturers and government authorities to evaluate the

allergenic potential and therefore potential hazards of latex products. This knowledge will help to make decisions whether to withhold certain latex products or batches with high allergen content.

The presence of latex allergens in the 43–46-kDa range was demonstrated by several investigators [6, 7, 10–12]. The high prevalence of IgE antibodies against these latex allergens ranging from 23% [12] to 80% [6] and their presence in ammoniated, non-ammoniated latex and manufactured latex products made these allergens especially interesting targets for diagnosis of latex allergy and monitoring of allergen content in consumer products. Recently, Beezhold et al. [11, 12] reported a 46-kDa latex protein that bound IgE from sera of 9/40 (23%) latex-allergic health care workers in IgE immunoblots. Two peptide sequences derived from this 46-kDa latex allergen revealed sequence similarity to patatin, a storage protein from the Solanaceae family [13]. The molecular characterization of this patatin-like latex allergen could help explain the molecular basis for the high incidence of potato allergy in the latex-sensitized population [11].

Here we report the Hev b 7 cDNA sequence coding for a 43-kDa allergen with sequence similarity to patatins. Recombinant Hev b 7 was produced in the methylotrophic yeast *P. pastoris* and displayed esterase activity. The IgE-binding capacity of the natural and recombinant protein was tested by IgE immunoblots with sera from latex-sensitized patients.

MATERIALS AND METHODS

Preparation of latex protein extracts. Latex was collected into chilled containers without ammonification and centrifuged

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Abbreviations. RAST, radioallergosorbent test; NRL, natural rubber latex; PNGase F, peptide N-glycosidase F; pfu, plaque-forming units.

Enzyme. Peptide N-glycosidase F (EC 3.5.1.52).

Note. The nucleotide sequence reported in this paper has been submitted to the EMBL database and assigned the accession number AJ223038.

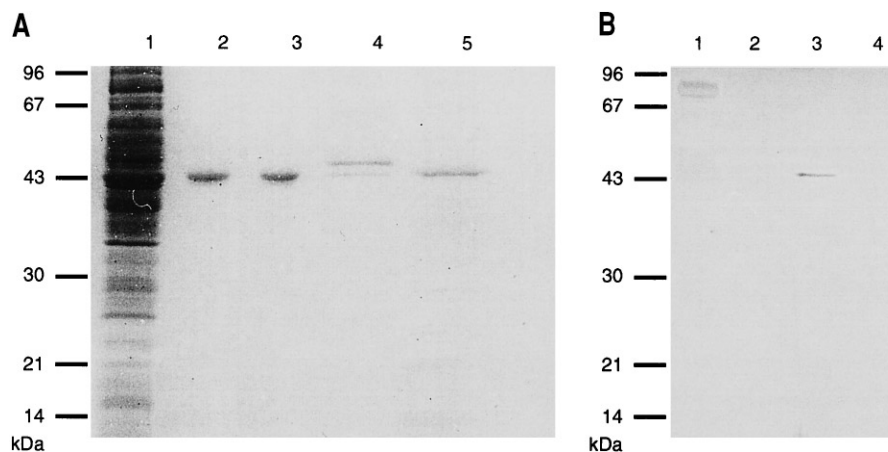


Fig. 1. SDS/PAGE and carbohydrate analysis of natural and recombinant Hev b 7. (A) Coomassie-stained SDS/PAGE. Lane 1, latex C-serum proteins; lane 2, natural Hev b 7 purified from latex C-serum; lane 3, purified natural Hev b 7 treated with PNGase F; lane 4, recombinant Hev b 7 purified from the culture supernatant of *P. pastoris*; lane 5, recombinant Hev b 7 purified from the culture supernatant of *P. pastoris* treated with PNGase F. (B) Detection of carbohydrate moieties on Hev b 7 by DIG glycan detection kit. Lane 1, human transferrin (80 kDa) as positive control; lane 2, natural Hev b 7 purified from latex C-serum; lane 3, recombinant Hev b 7 purified from the culture supernatant of *P. pastoris*; lane 4, recombinant Hev b 7 purified from the culture supernatant of *P. pastoris* treated with PNGase F. Protein quantities loaded were the same as in A.

at $40\,000\times g$ for 2 h, after which the latex separated into three main fractions: the rubber cream at the top, the bottom fraction, and the aqueous C-serum in between. The clear aqueous C-serum was freeze-dried immediately. The lyophilized non-ammoniated latex proteins were reconstituted at 10 mg/ml in NaCl/P₈ prior to use for SDS/PAGE and immunoblots.

Purification and sequence analysis of natural Hev b 7.

The purification of natural Hev b 7 from latex C-serum was carried out as described elsewhere [14]. The purified natural Hev b 7 was digested with CNBr, trypsin and endoproteinase-lys-C according to standard protocols [15]. Peptides were isolated by reverse-phase HPLC on a Nucleosil 120-5C18AB column, with a gradient of 0–70% acetonitrile in 0.1% CF₃COOH. The sequences of the peptides and the full-length protein were determined by automated Edman degradation and analysis on a 477A gas-phase microsequencer (Applied Biosystems) connected on-line to the phenylthiohydantoin analyzer, model 120 A.

RNA preparation. For the isolation of RNA, 200 ml latex was collected by tapping a *Hevea brasiliensis* clone RRIM 600 tree. During the collection, the latex was continuously mixed with an equal volume of RNA extraction buffer (0.1 M Tris/HCl, 0.3 M LiCl, 0.01 M EDTA, 10% SDS, pH 9.5). This mixture was centrifuged at $100\,000\times g$ for 30 min at 15°C. The rubber plug was then removed and total RNA precipitated with LiCl after extracting the aqueous phase with CHCl₃/phenol. Poly(A)-rich RNA was obtained by using the PolyATtract[®] mRNA isolation system (Promega Corporation).

Preparation of the hybridization probe. Total RNA from *S. tuberosum* cv. Bintje was isolated as described previously [13]. 1 µg total RNA was reverse transcribed into cDNA and the hybridization probe was amplified by PCR using the oligonucleotide primers 5'-ACTGTTCTAAGTATTGATGGAG-3' (sense) and 5'-AAAAATATGAGGGCCATGTTTCG-3' (antisense) and labeled with [³²P]dCTP by the random primed DNA labeling kit (Boehringer Mannheim). The labeled 255-bp fragment corresponded to bases 88–342 of the *S. tuberosum* patatin gene B2 [13] (EMBL X13178).

Construction and screening of the cDNA library. Double-stranded cDNA was prepared from 5 µg poly(A)-rich RNA with the λ ZAP Express cDNA synthesis kit (Stratagene) and ligated into the λ ZAP II vector with EcoRI linkers. The ligation product was then packaged *in vitro* by using the Gigapack II Gold pack-

aging system (Stratagene). The cDNA insert size was greater than 400 bp and the library comprised 1.2×10^6 phages before amplification. For screening of the latex cDNA library the *Escherichia coli* strain XL1 Blue was infected with 10^6 pfu of independent recombinant phage and plated at a density of 30 000 pfu/150-mm plate. Plaques were transferred to Nylon Nyntran membranes (Schleicher & Schuell). The filters were hybridized and then the heterologous patatin cDNA probe was added at 6×10^5 cpm/ml hybridization solution and incubated for 16 h at 42°C. Plaques producing positive signals were selected and rescreened. R408 helper phage was used to rescue pBluescript plasmids. The isolated plasmid DNA was purified and sequenced.

DNA sequencing. Sequence analysis was performed using the Thermosequenase fluorescent labeled primer cycle sequencing kit (Amersham Life Science) and the LI-COR DNA sequencer model 4000L. Both strands were analyzed to yield the sequences.

Computer search for sequence similarity. The FASTA program provided with the Wisconsin Package (Genetics Computer Group, Madison WI) was used to search for protein sequence similarities of the 43-kDa latex allergen to proteins in the SWISS-PROT database.

Expression of recombinant Hev b 7 in the yeast *P. pastoris*. In the first approach, the full-length cDNA of Hev b 7 was amplified by PCR using the primers 5'-ATTAAGGATCCAAAC-CATGGCTACTGGTAGTACTACTTTTGAC-3' (priming region underlined, *Bam*HI site in italics) 5'-TTAGCGGCCGCTTAT-CATTTGAGTTGACGGAGC-3' (*Not*I), the PCR product cut with *Bam*HI and *Not*I and ligated into the *Bam*HI/*Not*I-digested *P. pastoris* expression vector pPIC3 (Invitrogen) for expression as a non-fusion protein. In the second approach, the Hev b 7 cDNA was fused in-frame to the *Saccharomyces cerevisiae* mating factor α leader peptide present in the *P. pastoris* expression vector pPIC9 (Invitrogen). In the latter case the N-terminal Met was mutated to Gly as a cloning artefact. Primers used for the second construct were 5'-ATTATCTCGAGAAAAGAGGGGC-TACTGGTAGTACTACTTTTGAC-3' (*Xho*I) and the same antisense oligonucleotide as given above. The *Xho*I/*Not*I-digested PCR product was ligated to the respective sites of the *P. pastoris* vector pPIC9 used for extracellular expression. The transformation of the *P. pastoris* strain GS115 (Invitrogen), screening for

Fig. 2. Protein and peptide sequence alignment. Peptides obtained by endoproteinase-lys-C (2), cyanobromide (3) and tryptic (5) digestion from purified natural Hev b 7 were compared to the deduced amino acid sequence of Hev b 7 and sequences of patatins from potato (*S. tuberosum*, SWISS-PROT P15477) and tobacco (*N. tabacum*, EMBL U68484). Peptides 1 and 4, see [11]. Dots indicate identical residues, asterisks indicate residues identical in all sequences compared.

Carbohydrate analysis and removal. To test for the presence of carbohydrate moieties the DIG glycan detection kit (Boehringer Mannheim) was used according to the manufacturer's instructions. To remove N-glycosidically linked carbohy-

SDS/PAGE and immunoblotting. Recombinant Hev b 7, natural Hev b 7 and latex C-serum proteins were analyzed by 12% SDS/PAGE under reducing conditions. The separated proteins were visualized by staining with Coomassie brilliant blue R-250 (BioRad), or transferred to Protran nitrocellulose mem-

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1  ATTTGCTATACGAGGACAGAGAATTAAGTTCTCTTGACAGCAAC
45  ATGGCTACTGGTAGTACTACTTTGACGCAAGGAAAGAAGATAACAGTGTAAAGCATTGAT
    M A T G S T T L T Q G K K I T V L S I D
105 GGAGGTGGGATTAGAGGGATCATTCCTGGTATCATTCTTGCTTCCCTTGAATCCAAGCTT
    G G G I R G I I P G I I L A S L E S K L
165 CAGGATCTGGATGGACCAGATGCAAGAATTGCAGATTATTTTGATATAATTGCAGGGACA
    Q D L D G P D A R I A D Y F D I I A G T
225 AGTACCGGTGGGCTGATAACCACCATGCTTACAGCTCCAAACGAGGATAAAAAACCTATG
    S T G G L I T T M L T A P N E D K K P M
285 TACCAAGCAAAAGACATAAAGGACTTTTATTGGAAACTGCCCAAGATTTTCCCAAAG
    Y Q A K D I K D F Y L E N C P K I F P K
345 GAAAGTCGAGATAATTACGATCCTATCCATTCAATAGGACCCATTTATGATGGAGAATAT
    E S R D N Y D P I H S I G P I Y D G E Y
405 CTGCGAGAATTGTGTAACAATTTGCTCAAGGACTTGACCGTAAAAGATACCTTGACAGAT
    L R E L C N N L L K D L T V K D T L T D
465 GTGATTATACCTACATTTGACATCAAGCTTCTTCTCCAGTCATCTTCTCATCTGATGAT
    V I I P T F D I K L L L P V I F S S D D
525 GCGAAATGTAATGCTCTGAAAAATGCTAGGCTAGCTGATGTCTGCATCAGCACCTCTGCA
    A K C N A L K N A R L A D V C I S T S A
585 GCTCCAGTTCTCTTACCAGCACATTCCTTTACCACCGAGGATGATAAAAAATATTCACACT
    A P V L L P A H S F T T E D D K N I H T
645 TTTGAACTCATTGATGGTGGGCTGCTGCGACCAATCCTACACTACTAGCACTAACCAC
    F E L I D G G A A A T N P T L L A L T H
705 ATCCGCAACGAGATCATTAGGCAAAATCCTCGGTTCAATGGTGCAAACCTTGACGGAGAGC
    I R N E I I R Q N P R F I G A N L T E S
765 AAGAGCAGGCTAGTGCTATCACTGGGAACTGGCAATCCGAGTACAAAGAAAAATACAAT
    K S R L V L S L G T G K S E Y K E K Y N
825 GCAGACATGACATCTAAATGGCGTTTGTATAATTGGGCCCTTTACAACGGCAATTCACCA
    A D M T S K W R L Y N W A L Y N G N S P
885 GCGGTTGACATATTTAGTAATGCTAGCTCAGATATGGTTGACTTCCATCTCTCTGCCCTT
    A V D I F S N A S S D M V D F H L S A L
945 TTTAAGTCCTTGATTGTGAGGATTATTACCTGCGCATTACAGATGATACGCTGACGGGT
    F K S L D C E D Y Y L R I Q D D T L T G
1005 GAAGAATCATCAGGTCATATAGCAACCGAGGAGAATTTGCAAAGGCTTGTGGAATTTGGA
    E E S S G H I A T E E N L Q R L V E I G
1065 ACTAGGCTCCTGGAGAAGCAGGAGTCAAGGATTAATTTGGATACTGGTAGGCTTGAAAGC
    T R L L E K Q E S R I N L D T G R L E S
1125 ATTCCAGGGGCTCTACTAATGAAGCAGCTATAACTAAATTTGCAAAGCTGCTTTCAGAG
    I P G A S T N E A A I T K F A K L L S E
1185 GAACGAAAGCTCCGTCAACTCAAATGAGGCAAATTTCTGCCACAATTCATGGACTTAA
    E R K L R Q L K *
1245 CACAATATGCATAATAAAATACTATAAAATAAATTTGCATCTTAATTTTCATTATGAATCGA
1305 ACTTAAGTGAGCTTGTTCCTTAAATAATAAGTTTGTGAGCTTGTGACACTTTATTG
1365 TAAAATATTAATAATGTCAATGTGGCCTTAAAGGTCAATTTAGCTTCTAAATCGAAAAA
1425 AAAAAAAAAAAAAAAAAAAAAA

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Fig. 3. Nucleotide sequence and deduced amino acid sequence of Hev b 7, the 43-kDa latex allergen. Consensus sequences for N-glycosylation are underlined and asparagine residues shown in bold. Two putative polyadenylation signals are underlined.

branes (Schleicher & Schuell) for immunodetection. IgG blots were performed with rabbit anti-(Hev b 7) serum diluted 1:200. IgE-binding ability of proteins was detected with sera from allergic patients and bound IgE detected using ¹²⁵I-labeled rabbit anti-human IgE (RAST RIA, Pharmacia) diluted 1:10. For inhibition of IgE binding to latex C-serum, four individual serum

samples were incubated overnight at 4°C with 10 µg purified recombinant Hev b 7. The incubated sera were used to probe the nitrocellulose strips containing latex C-serum proteins.

Patients. A total of 36 individual serum samples from patients with positive case histories, positive skin prick tests, positive radioallergosorbent tests (RAST classes >2, mean value 3)

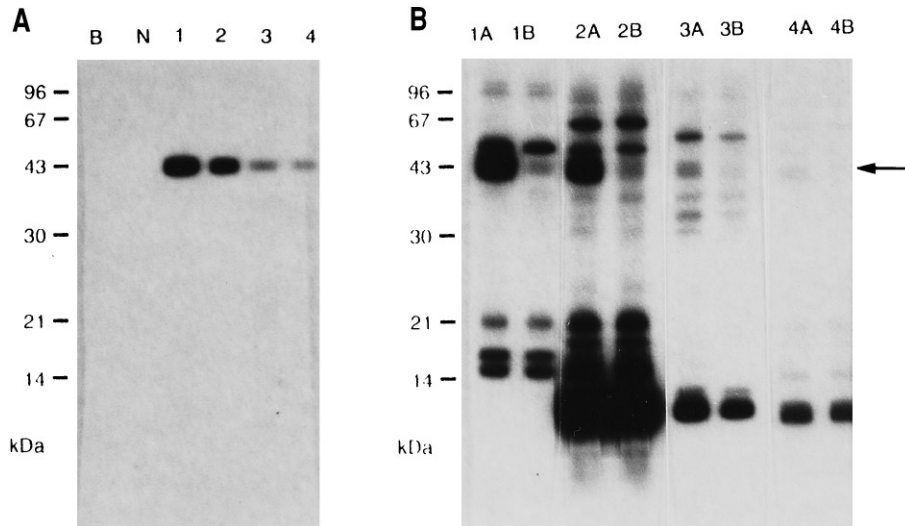


Fig. 4. IgE immunoblots and inhibition experiments. (A) IgE immunoblot. Blotted recombinant Hev b 7 was probed with sera from latex-allergic patients (lanes 1–4), with a normal human serum pool (lane N) or buffer control (lane B). Bound IgE was detected with ^{125} I-labelled rabbit anti-human IgE. (B) Inhibition experiments. Blotted latex C-serum proteins were probed with the same sera as in A (lanes A). Inhibitions were performed by incubating sera first with 10 μ g recombinant Hev b 7 per lane (lanes B). The arrow indicates the position of Hev b 7.

and characteristic type-I allergic reactions to latex were used in this study. A serum pool from 22 healthy individuals with no histories of any type-I allergy, negative skin prick tests, and negative RAST results to latex allergens was used as negative control.

RESULTS

Purification and analysis of natural Hev b 7. The purified natural Hev b 7 was calculated to make up 0.3% of the total protein of C-serum proteins and was homogeneous (Fig. 1A, lane 2). The N-terminus of the natural Hev b 7 was blocked. Internal amino acid sequences were therefore determined following digestion with CNBr (LTAPNEDKKPM), trypsin (DNYDPIHSIGPIYDGXYLR) and endoproteinase-lys-C (ITVLSIDGGGIXGIIXGIIILASLES). The alignment of these peptides with the deduced amino acid sequence of Hev b 7 is shown in Fig. 2.

Isolation and characterization of Hev b 7 cDNA clones. The analysis of three positive clones identical in their coding and 3' untranslated regions yielded the sequence shown in Fig. 3. The Hev b 7 cDNA comprised 1444 bp and had an open reading frame of 1164 bp which encoded a polypeptide of 388 amino acid residues with a predicted molecular mass of 42 871 Da and a calculated pI of 5.0. Two potential N-glycosylation sites were found (Fig. 3). An analysis of the deduced amino acid sequence of Hev b 7 with the program SIGSEQ2 [17] revealed the absence of an N-terminal leader peptide.

Sequence similarity analysis. The FASTA search algorithm implemented in the Wisconsin Package revealed that Hev b 7 shared substantial sequence similarities with patatins from *S. tuberosum* and *N. tabacum*; 43% identity with *N. tabacum* patatin (in a 388-amino-acid overlap) and 39–42% with different patatins from *S. tuberosum*.

Expression of recombinant Hev b 7 in the yeast *P. pastoris* and its purification. The expression using the pPIC3 vector yielded a single band of 43 kDa in the cellular fraction recognized by rabbit anti-(natural Hev b 7) serum (data not shown).

Using pPIC9, two bands differing by 4–5 kDa in molecular mass were detected (Fig. 1A, lane 4). To confirm the identity of the secreted recombinant proteins the first 12 amino acids of the two bands were sequenced yielding the same peptide sequence, GATGSTTLTQK, which corresponds to the N-terminus of the recombinant Hev b 7. Both bands were detected by the rabbit antiserum showing that they were immunologically equivalent (data not shown). When purified over a Resource Q column, the protein eluted at 125–150 mM NaCl. After PNGase F treatment and a second purification step using a phenyl-Sepharose CL-4B column as described for natural Hev b 7, the protein appeared to be homogeneous, as determined by SDS/PAGE (Fig. 1A, lane 5). The purified recombinant Hev b 7 was totally soluble in aqueous solutions and its yield was calculated to be 10 mg/l culture supernatant.

Carbohydrate analysis. The treatment of natural Hev b 7 with PNGase F, which cleaves N-glycosidically linked glycans, did not change the electrophoretic mobility of purified natural Hev b 7 (Fig. 1A, lanes 2 and 3). In addition, a carbohydrate analysis performed with the DIG glycan detection kit showed the absence of carbohydrates (Fig. 1B, lane 2). To test the possibility that the two forms of secreted recombinant Hev b 7 represented the glycosylated and non-glycosylated form of the same peptide or products of C-terminal degradation we performed a PNGase F treatment of the purified double band. After the treatment only the lower-molecular-mass band remained (Fig. 1A, lane 5). Using the DIG glycan detection kit, we could confirm that only the higher-molecular-mass band was glycosylated. Thus we could clearly show that the two bands in the culture supernatant of *P. pastoris* are the glycosylated and non-glycosylated forms of the same polypeptide.

Esterase activity. The specific activity of the purified recombinant Hev b 7 determined for *p*-nitrophenyl palmitate was 0.508 ± 0.006 U/mg protein or, expressed as $\Delta A_{400}/9.405 \pm 0.120/10 \text{ min} \times (\text{mg recombinant Hev b 7 protein})^{-1}$. This value is higher than specific activities of two natural patatin isoforms purified from *S. tuberosum* patatins, which displayed ΔA_{400} of 1.5 and $0.25/10 \text{ min} \times (\text{mg protein})^{-1}$ [18].

Immunoblots and inhibition experiments. Immunoblot analyses of latex C-serum proteins, of natural and recombinant Hev b 7 were performed with sera from individuals allergic to NRL. Natural Hev b 7 and recombinant Hev b 7 were equivalent in their ability to bind IgE (data not shown). IgE immunoblots with serum samples containing IgE against Hev b 7 are shown in Fig. 4A, lanes 1–4. Hev b 7 was able to bind IgE from 4/36 sera (11%). No IgE-binding could be detected with the pool of sera from non-allergic individuals (Fig. 4A, lane N). Using 10 µg recombinant Hev b 7 the IgE binding to natural Hev b 7 present in latex C-serum could be partially inhibited (Fig. 4B).

DISCUSSION

The construction of the *H. brasiliensis* latex cDNA library allowed us to characterize the 43-kDa latex allergen Hev b 7 in great detail. We cloned Hev b 7 by screening the cDNA library with a 255-bp fragment of *S. tuberosum* patatin gene B2 [13]. The hybridization probe was chosen to comprise a region of *S. tuberosum* patatin B2, to which four peptides derived from natural Hev b 7 could be aligned best (peptides 1–4 in Fig. 2).

The Hev b 7 cDNA encodes a latex protein of 388 amino acid residues with a predicted molecular mass of 42 871 Da and a calculated pI of 5.0 (Fig. 3). The protein had 39–42% identity with different patatins from *S. tuberosum* (Fig. 2). Three internal peptides derived from the purified natural Hev b 7 matched the deduced amino acid sequence of Hev b 7 with the exception of one amino acid (Fig. 2). Peptide 1 in Fig. 2 reported by Beezhold et al. [11] as the N-terminus of the patatin-like protein is located 7 amino acid residues downstream from the beginning of the ORF of Hev b 7. The N-terminus of the natural Hev b 7 purified in this study was blocked.

Patatin is the trivial name given to a family of glycoproteins that make up over 30% of the total soluble protein in potato tubers [19]. Esterase, lipid acyl hydrolase and acyltransferase activities were unambiguously shown for the recombinant form of a *S. tuberosum* patatin expressed in the baculovirus system [16]. It has been shown that members of the patatin protein family from potato become N-glycosylated, contain a signal peptide and are localized in the vacuoles [20–22].

A surprising result was the lack of a leader peptide at the N-terminus of the natural Hev b 7 protein indicating that cytoplasmic localization of the protein should be taken into consideration. Two potential N-glycosylation sites were found in the deduced amino acid sequence of Hev b 7 (Fig. 3). However, the natural Hev b 7 was obviously not glycosylated as shown by PNGase F treatment (Fig. 1, lanes 2 and 3) and the use of DIG glycan detection kit (Fig. 1B, lane 2). On the other hand, the glycosylation signals were functional as we could clearly show by fusing the full-length coding region of Hev b 7 to a yeast leader peptide and secreted expression in the yeast *P. pastoris*. The analysis of secreted recombinant Hev b 7 showed that the major part of the protein was N-glycosylated (Fig. 1A, lanes 4 and 5, Fig. 1B, lanes 3 and 4). In line with the lack of a leader peptide for transit into the endoplasmic reticulum, these data support the idea of the cytoplasmic localization of Hev b 7. The patatin-like Hev b 7 would, in contrast to vacuolar patatins from *S. tuberosum*, have cytoplasmic localization. These data are consistent with the proposed function of Hev b 7 as an inhibitor of rubber biosynthesis, which takes place in the cytoplasm [14, 23]. The efficiency of the extracellular *P. pastoris* expression system was 10 mg recombinant Hev b 7/l culture. The expression of the coding region of the recombinant Hev b 7 as non-fusion protein resulted in cellular localization of the protein (data not shown). For the ease of purification, we used the secreted expression to

purify milligram quantities of recombinant Hev b 7. The purified protein displayed, similarly to patatins from *S. tuberosum*, esterase activity indicating that the recombinant protein is correctly folded and equivalent to its natural counterpart, not only in immunological terms (IgE binding) but also with respect to biochemical features.

We tested the IgE-binding capacity of the recombinant Hev b 7 by IgE immunoblots using 36 individual serum samples from latex-allergic patients. IgE from sera of 4 patients (11%) bound to the recombinant Hev b 7 (Fig. 4A, lanes 1–4) and natural Hev b 7 (data not shown). Despite the fact that Hev b 7 is a minor allergen, we could show that it was an important allergen for some patients (Fig. 4B, lanes 1A and 1B). The much higher percentage of IgE reactivity in the 43–46-kDa range described in the literature [6, 7, 10–12] could be due to geographic differences in NRL sensitization or simply due to the presence of other, so far uncharacterized allergens in the same molecular mass range. The IgE reactivity to 43-kDa proteins from latex C-serum could be inhibited by prior incubation of the sera with 10 µg recombinant Hev b 7/lane, but the inhibition was only partial. This might be due to the presence of several Hev b 7 isoforms in *H. brasiliensis*. In *Solanum* species, eight different cDNAs coding for patatins have been identified [24]. One of the peptide sequences determined for natural Hev b 7 reported in this paper does not completely match the amino acid sequence deduced for Hev b 7 from the cDNA clones (Fig. 2), which is a first hint for the presence of Hev b 7 isoforms. In addition, a different molecular mass of 46 kDa for the patatin-like latex allergen has been reported by Beezhold and coworkers [11].

Because the Hev b 7 protein is only a minor component of latex C-serum and obtaining sufficient amounts of purified natural Hev b 7 is difficult and time-consuming, the recombinant Hev b 7 presents an economic alternative to the preparation from *H. brasiliensis* sap. For reliable, unequivocal diagnosis of latex allergy highly purified and standardized reagents containing the proteins responsible for IgE binding and IgE-mediated symptoms are essential. The difficulties in extracting, purifying and standardizing allergens from latex make them less suited for diagnostic and therapeutic purposes than their recombinant counterparts.

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