
Lipid transfer protein from *Hevea brasiliensis* (Hev b 12), a cross-reactive latex protein

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Background: Latex-allergic individuals experience clinical cross-reactivity to a large number of fruits and vegetables. Much of the cross-reactivity can be attributed to Hev b 6, but evidence indicates that additional cross-reactive allergens may be present. A common pan-allergen, which has not previously been identified in latex, but may contribute to this cross-reactivity is lipid transfer protein (LTP). We sought to determine whether *Hevea brasiliensis* produces LTP and whether it would bind immunoglobulin E from latex-allergic patients.

Methods: LTP was identified in *H. brasiliensis* RNA by polymerase chain reaction using degenerate primers. The entire cDNA was obtained by polymerase chain reaction using rapid amplification of cDNA ends reactions. The complete coding sequence for LTP was determined and produced as a recombinant protein using the glutathione *S*-transferase and pET32 expression systems. Immunoblot analysis of sera from latex-allergic patients was used to determine whether patients recognize LTP as an allergen.

Results: We identified a 662-basepair cDNA with a 351-basepair open reading frame that encodes for a 116-amino acid protein. The protein has significant homology to the family of nonspecific LTPs. We expressed the protein as a mature LTP of 92 amino acids with a predicted isoelectric point of 10.8 and molecular weight of 9.3 kDa. Immunoblots demonstrated specific immunoglobulin E for LTP in the sera of 9 of 37 (24%) latex-allergic individuals.

Conclusions: We describe the initial identification of rLTP in *H. brasiliensis* that may be important as a cross-reactive pan-allergen (Hev b 12).

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INTRODUCTION

Lipid transfer proteins (LTPs) form a family of approximately 9-kDa proteins that are widely distributed throughout the plant kingdom.¹ The proteins are characterized by eight conserved cysteines that form four disulfide bridges making a stable protein structure resistant to harsh temperatures and pH changes.² The proteins are members of the PR-14 family of pathogenesis-related proteins that have potent antifungal and antibacterial activities in plants.³ LTPs from a number of plants have been identified as allergens.^{4–8} LTPs constitute a common allergen of the Rosaceae family (apple, peach, apricot, plums, pears, etc) and represent the major allergen in birch pollen independent allergy to Rosaceae.⁴ Because LTPs from a variety of fruits and vegetables are highly conserved, cross-reactivity between the various LTPs is common and the protein family has been characterized as pan-allergen.⁹ The structural stability of LTP makes it resistant to harsh conditions and protease digestion making the protein one of the few barley proteins that can survive the brewing process and consequently is a major allergen in beer.^{9,10}

Latex-allergic patients experience symptoms ranging from contact urticaria to anaphylaxis following contact with latex

gloves or other latex-containing products.¹¹ Latex allergy involves multiple proteins from the *Hevea brasiliensis* tree that remain on latex medical products. Eleven latex proteins have been identified as allergens and given allergen designations (Hev b 1 to 11), many of which are homologous to protein allergens found in other plants. Sera from latex-allergic patients show a high degree of in vitro cross-reactivity with many plant proteins,¹² and patients experience symptomatic clinical cross-reactivity to certain foods primarily banana, avocado, and kiwi.¹³ Much of the food cross-reactivity has been attributed to the pan-allergen hevein (Hev b 6.02); however, not all of the cross-reactivity can be attributed to Hev b 6. Because latex-allergic patients report cross-reactivity to peach, apple, and other fruits,¹⁴ in which LTP is a major allergen, we sought to determine whether LTP was present in latex and whether it could bind immunoglobulin (Ig)E from latex-allergic patients. In this paper, we describe the LTP from *H. brasiliensis* that has been designated Hev b 12.

MATERIALS AND METHODS

RNA Purification

RNA was extracted from *H. brasiliensis* (cv. RRIM c600) leaves by cetyltrimethylammonium bromide and LiCl precipitation,¹⁵ and from latex by a modification of the method of Prescott and Martin¹⁶ as previously described.¹⁷

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First-Strand cDNA Synthesis and Polymerase Chain Reaction (PCR) of a Partial Sequence of LTP

Total RNA from Hevea leaf and latex (1 μ g) was reverse transcribed to cDNA using oligo (dT)18 and MMLV reverse transcriptase with the Advantage RT-for-PCR kit (BD Biosciences Clontech, Palo Alto, CA). Degenerate oligonucleotide primers were designed based on conserved regions of LTP using a strategy similar to that of Scheurer et al⁸ for cherry LTP. An Eco R1 site (italicized) was incorporated for cloning purposes. The primers used were: LTP latex 1F 5' degenerate primer (amino acids ITCGQV) CCG ATT GAA TTC ATH ACN TGY GGN CAR GT and LTP latex 1R 3' degenerate primer (amino acids LPGKCGV) CCG ATT GAA TTC ACN CCR CAY TTN CCN GGN AG (Invitrogen, Carlsbad CA) where R = A and G; Y = C and T, H = A, T, and C; and N = A, T, C, and G.

Rapid Amplification of 5' and 3' cDNA Ends

For amplification of the unknown 5' and 3' ends of latex LTP, the switching mechanism at 5' end of RNA transcripts (SMART) rapid amplification of cDNA ends (RACE) cDNA Amplification kit (BD Biosciences Clontech) was used according to the manufacturer's instructions. Total RNA (1 μ g) from Hevea leaf or latex was used in first-strand cDNA synthesis primed with 3' coding sequence (CDS) primer for 3' RACE-ready-cDNA, and primed with 5' CDS primer and SMART II A oligo for 5' RACE-ready-cDNA. The cDNA was amplified with the Universal Primer provided with the SMART RACE kit, and with gene-specific primers based on the determined latex LTP partial sequence: LTP 4R GAC CCG CTA CGG TGG TG for 5' RACE or LTP 6F CGC TCT CGT CCC ATG TC for 3' RACE.

Cloning and Sequencing of LTP cDNA

The PCR products of the correct size were purified from a 2% agarose gel (SeaKem GTG, Biowhittaker, Rockland, MD) using Nucleobond (BD Biosciences Clontech), ligated into pGEMTeasy (Promega, Madison, WI), and transformed in *Escherichia coli* JM109 cells. Positive clones were selected by using a blue/white screening. Plasmid preps were performed using the QIAprep Spin Miniprep kit (QIAGEN, Valencia, CA) followed by Eco R1 digestion to check for an insert. DNA sequence analyses was performed using an ABI PRISM 377 DNA sequencer (Applied Biosystems, Weiterstadt, Germany). Sequences were compared with the GenBank database using the Basic Local Alignment Search Tool (BLAST).

Coding Sequence of LTP

Based on the sequences obtained, PCR primers LTP forward 5' CCG ATGAATTCATGATAACATGTGGTCAAGTAC 3' and LTP reverse 5' CCGATGA ATTCTCACTTGACGGTG-GCACAGTTTG 3' were used to obtain the coding sequence of the mature LTP, omitting the signal peptide and including the stop codon. Eco R1 sites (italicized) were again included for cloning purposes. The PCR was performed and the product purified using the QIAquick PCR purification kit (QIAGEN), Eco R1 digested to remove the region of the primers

flanking the Eco RI site, and analyzed on a 2% agarose gel (SeaKem GTG). The band was excised from the gel and purified with QIAEX II Gel Extraction kit (QIAGEN).

Expression of Recombinant LTP

To produce recombinant LTP, the PCR product for mature LTP including a stop codon was ligated into two different *E. coli* expression systems. The glutathione S-transferase (GST) expression system provides a GST tag for easy purification and convenient cleavage of the vector using thrombin. We also used the versatile pET vector system to adjust for any potential bactericidal effect of LTP or to enhance the formation of disulfide bonds. The 166-amino acid pET expression region contains a 109-amino acid portion of thioredoxin (Trx-tag) followed by His-tag and S-tag sequences. A cleavage site for thrombin is found between the His-tag and the S-tag, and for enterokinase between the S-tag and the multiple cloning site. Thrombin cleavage results in 39 vector-derived amino acids before the expressed protein, whereas enterokinase leaves nine amino acids. The Trx-tag promotes the formation of disulfide bonds when expressed in TrxB-negative mutant host strains, while expression in pLysS host allows for tighter control of expression for toxic proteins.

For GST/LTP expression, the LTP PCR product was ligated into the EcoR1 site of the pGEX-4T-1 vector (Amersham Biosciences, Piscataway, NJ) and expressed in BL21 *E. coli* as previously described for Hev b 5.¹⁸ The GST/LTP fusion protein was isolated from bacterial sonicates over a glutathione column (Amersham Biosciences) according to the manufacturer's instructions and the GST tag removed by thrombin cleavage, resulting in a 9.3-kDa mature LTP protein beginning with five amino acids derived from the vector (GSPEF).

The LTP PCR product was also ligated into the EcoR1 site of the pET32a plasmid (Novagen, Madison, WI), and transformed into *E. coli* BL21 Gold (DE3) pLysS (Amersham Biosciences) for toxic proteins because of the potential bactericidal activity of LTP and into the TrxB-negative strain AD494(DE) for promoting the formation of the four disulfide bonds in LTP. The pET32a/LTP fusion proteins were isolated from bacterial sonicates by affinity chromatography over a Ni-NTA column (QIAGEN). The fusion protein was left intact or cut with thrombin (10 U/mg, overnight) or enterokinase (20 U/mg, overnight) according to the manufacturer's instructions. For a control, pET32 (Induction Control J, Novagen) was expressed in BL21 (DE3) producing a recombinant thioredoxin with attached purification tags (approximately 20 kDa) and purified with Ni-NTa resin as above. Total protein concentrations were determined using the Coomassie protein assay (Pierce, Rockford, IL).

Anti-LTP Sera

Sera from 37 latex-allergic patients were screened for IgE reactivity to Hev b 12 by Western blot. The sera were obtained from latex-allergic patients who were symptomatic with exposure to latex and had positive latex skin prick tests.

Latex-related symptoms of 29 of the 37 patients were reported previously.¹⁹ Sera from 15 atopic non-latex-allergic patients also used in our previous studies served as controls.¹⁹ The rabbit sera from three animals immunized with rLTPs from peach and carrot, or native LTP from apple, and sera from two patients with IgE to fruit LTP were provided by R. van Ree.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Immunoblot

The LTP proteins (1 µg/lane) were separated by SDS-PAGE on 15% gels under reducing conditions²⁰ and non-reducing conditions. For immunoblots, the pET32/LTP fusion protein was transferred to nitrocellulose (0.1 µm; Schleicher & Schuell, Keene, NH) and reacted with patient sera (1/10 dilution) or rabbit anti-LTP (1/3,000). IgE binding was detected with a monoclonal anti-human IgE (Clone GE-1, Sigma, St. Louis, MO) diluted 1/1,000 and an alkaline phosphatase labeled goat anti-mouse IgG (Promega) diluted 1/5,000, whereas rabbit IgG was detected with an alkaline phosphatase labeled anti-rabbit IgG (Promega) as previously described.²¹

RESULTS

H. brasiliensis LTP cDNA

Primers, designed according to conserved sequences in other known LTPs, were used to screen the *Hevea* cDNA from latex and leaf material. Initial screening resulted in amplification of a 185-basepair (bp) product from both leaf and latex RNA. The products from both sources were sequenced and found to be identical. Using *Hevea* leaf and latex RNA, 5' and 3' RACE were used to obtain the complete cDNA se-

quence for *Hevea* LTP (Fig 1). Five different clones of both leaf and latex 5' and 3' RACE reactions were sequenced and aligned using Assemblylign software (ABI, Foster City, CA) to determine the consensus sequence (data not shown). Sequences from the 10 5' RACE clones were nearly identical. Two of the clones from latex were truncated at position 5 and 58, a single bp change in two clones from leaf at position 277, and a single change at position 86 and 301 in clones from latex (Fig 1). One 3' clone had a change at position 241. Some heterogeneity, typical of plant cDNA,²² was observed in the 3' untranslated region beyond position 630. The entire sequence of *Hevea* LTP cDNA was 662 bp in length with the open reading frame identified to be 351 bp in length and coding for a protein of 116 amino acids. Based on the homology to other LTPs, the first 24 amino acids were predicted to be the signal peptide. Only one of the sequence changes resulted in a change in amino acid (F to S) at position 9 of the signal peptide. The consensus sequence of latex LTP is available through GenBank, accession number AY057860.

Latex LTP Protein and Homology to Other LTPs

The mature protein for *Hevea* LTP has a calculated molecular weight of 9.34 kDa and a theoretical isoelectric point (pI) of 10.8. Amino acid sequence alignment using BLAST revealed significant homologies to the family of nonspecific LTPs (Fig 2). The highest homology was to apple LTP (Mal d 3) with 69% identities and 82% conserved amino acid changes, but homologies to many fruit LTPs ranged from 57 to 69% identities (Fig 2). The most homologous regions appeared to be the first six amino acids and the C-terminal 25 amino acids. The eight cysteines, which form the four-disulfide bonds that characterize the LTP family, were also conserved

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1  ACCCACA CTGAAACCTTCATATATACCAAGAAATGTTGTGCTCTTTACAATTATCATC
   *
1  M A A L K M V S F L V L C M L V A A P M
61  ATGGCCGCACCTTAAGATGGTTAGCTTTCTTGTCTATGCATGTTGGTGGCAGCTCCAATG
   C
21  T A O A I T C G Q V Q S A L V P C L S Y
121 ACTGCACAAGCTATAACATGTGGTCAAGTACAAGCGCTCTCGTCCCATGTCTTAGTTAC

41  L K T T G P T P P A T C C N G V R T I N
181 CTCAAGACTACGGGCCAACCCCTCCGGCAACATGCTGTAATGGGTGAGGACCATCAAC

61  N A A K T T A D R R T A C Q C L K S A A
241 AACGCTGCTAAAACACCGCTGACCGCCGAAGTGTGTCAGTGCTTGAAATCAGCTGCT
   G C
81  G S V K G L N P T T V A G L P G K C G V
301 GGCAGCGTCAAGGGGCTTAACCCACACCGTAGCGGTCTCCCTGGTAAATGCGGAGTT

101 N I P Y K I S L S T N C A T V K -
361 AACATTCTCTACAAGATCAGCTGTCCACAACTGTGCCACCGTCAAGTGAGGTGCATAG

421 AGCGGGATTTTGGGTCCAGAGATGGTATAGTTTGGGAAGAATAATAAAGGCTCATCA

481 GCTGTGTGTAGTTTCAAGTGAACAGAGTCTCTGTCTCCACTTTAGCGAGGCTCCTTCTTC

541 TTACCTAGTGAATTTTTTTCAGTTTAGTCTCTTTTGTGTGGCTGTCTTTGGGCCCTTTCT

601 GCCTCCTGTGTCATAGATGAGATCCATATAAGTCTATCTTTTATATAAAAAAAAAAAAAA

661 AA

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Figure 1. *Hevea* LTP cDNA and protein sequence. The complete cDNA sequence of 10 5' RACE and 10 3' RACE products were aligned. An asterisk indicates points of truncated sequence for two of the 5' clones. In the open reading frame only four single nucleotide changes in five clones were found and are indicated below the sequence. Additional heterogeneity in the 3' end beyond position 630 is not indicated. Only one change (position 86) would result in an amino acid change from F to S. The underlined amino acids indicate the signal peptide.

Plant	gi#	Sequence	
Latex	20135538	ITCGQVQSALVPCLSYLKTGTGTP-PATCCNGVRTINNAAKTTADRRITACQCLKSAAGSV	59
Apple	14423814	*****T*S.A**IG*VRSG*AV*---****I***GL*****Q***N***NL***I	82
Apricot	7404406	*****S*S.A**IG*VRGG*AV*---****I*NV*L*R***P*****Q***N***QLS**I	58
Plum	9297015	*****S*N.A**IN*V.GG*AV*---****I*NV*L*R***P*****A***N***QLS**I	58
Strawberry	18477856	*****A*NIS***T*V*SG*AV*---A**S*I*NL*GM*****QA***N***Q***GI	85
Almond	2497752	*****S*N.A**IP*VRGG*AV*---****I*NV*L*R***P***QA***N***QLS**A	84
Peach	3287877	*****S**A**IP*VRGG*AV*---****I*NV*L*R***P***QA***N***QLS**A	58
Cherry	14423815	L*****S*N*A**IA*VRGG*AV*---****I*NV*L*****Q***N***QLS**A	84
Pear	14423813	***S**SAN*A**IN*VRSG*AV*---****I*NV*L*****Q***N***QLS**A	82
Chickpea	3914136	*****R*DT**A***G**Q-G**G*-S*Q**G**NL*S**V**P***QA***N*****I	82
Carrot	128384	L*****TG**A***G**RSQVNV*V*L***V*GL*****R*L*K*****G***QT.NA*	87
Tobacco	417371	LS*****G*A***P**QGR**L---GS**G**KGLLG**SLS**K***T*****NAI	81
Latex	20135538	KGLNPTTVAGLPGKCGVNIPIYKISLSTNCATVK	92 Identity/conserved
Apple	14423814	S*V**NNA*****V*****T*****	115 69%/82%
Apricot	7404406	S*V**NNA*A*****A*****	91 67%/77%
Plum	9297015	P*V**NNA*A*****V*****A*****	91 67%/77%
strawberry	18477856	*****NLA*****SV*****TT***A**	117 65%/78%
Almond	2497752	P*V**NNA*A*****Q**P*****N**	117 63%/73%
Peach	3287877	P*V**NNA*A*****H*****A*****	91 65%/75%
Cherry	14423815	P*V**ANNA*A*****V*****P*****	117 66%/76%
Pear	14423813	S*V**GNAES*****V*****T*****	115 64%/77%
Chickpea	3914136	SR**ANNA*A*****V*****T*****IR	115 65%/74%
Carrot	128384	T***LNAA****AR*****PT*D*NR*	119 60%/70%
Tobacco	417371	**IDMGKA*****A*****P**D*SK*Q	114 57%/74%

Figure 2. Homology of *Hevea* LTP with nonspecific LTPs from other plants. The eight conserved cysteines are indicated by underlining. An asterisk indicates amino acid identity, whereas a dash indicates a gap introduced to maximize the alignment.

in the *Hevea* LTP. One potential N-linked glycosylation site is located at position 87 to 90 (NPTT). Homology modeling was performed using Swiss-Model and Swiss-Pdb Viewer programs (Swiss Institute of Bioinformatics, Basel, Switzerland) and the structure of LTP from maize as a template. The model was highly similar to maize LTP and predicts that the four disulfide bonds occur at Cys 3-Cys 51, Cys 13-Cys28, Cys29-Cys74, and Cys 88-Cys 49.

Recombinant LTP

The coding sequence for the mature *Hevea* LTP was expressed in several bacterial expression systems. In the GST expression vector, a 39-kDa fusion protein was produced and

when cut with thrombin released the 9.5 kDa LTP (Fig 3). Expression of pET32a/LTP fusion protein in both pLysS and TrxB-negative bacteria produced a recombinant protein of approximately 30 kDa that was purified using the 6X His tag over the Ni column (Fig 3). Cutting the fusion protein with thrombin produced a doublet of protein migrating at approximately 15 kDa. Cutting with enterokinase resulted in an 18-kDa vector protein and a 10-kDa LTP (Fig 3).

The recombinant *Hevea* LTP was tested with rabbit antisera generated to rLTP from peach and carrot or native LTP from apple. Rabbit anti-apple LTP recognized the pET32a/LTP from *Hevea*, but not the pET32 vector control (Fig 4A). A similar pattern of reactivity was observed with the anti-peach and anti-carrot LTPs. Only the anti-carrot LTP could recognize *Hevea* LTP when it was cut from the fusion protein (data not shown). The rabbit anti-LTP activity could be completely inhibited with pET32a/LTP and with a 9.5-kDa *Hevea* LTP purified from a GST/LTP fusion protein cut with thrombin but not with the pET32a control (Fig 4B) demonstrating the rabbit antibodies were recognizing the LTP and not a vector component. Both the anti-apple and anti-peach LTP also recognized a 68-kDa band in ammoniated latex protein (Fig 4A), but this reactivity could not be inhibited with *Hevea* LTP.

IgE Immunoblots

Because rabbit anti-LTP demonstrated that the pET32a/LTP construct had an immunologically active LTP, *Hevea* LTP was left as a fusion protein for IgE immunoblot analysis. Immunoblots using sera from latex-allergic patients demonstrated IgE binding to the pET32a/LTP fusion protein (Fig 5). Of 37 latex-allergic patient sera tested, nine (24%) had a positive reaction demonstrating specific IgE to LTP. Of the 9 Hev b 12 positive patients, 2 reported clinical reactivity to fruits of the Rosaceae family, and fruit reactivity was not noted in the charts for the other 7 patients. IgE from control patients did not bind to *Hevea* LTP. In some latex-allergic

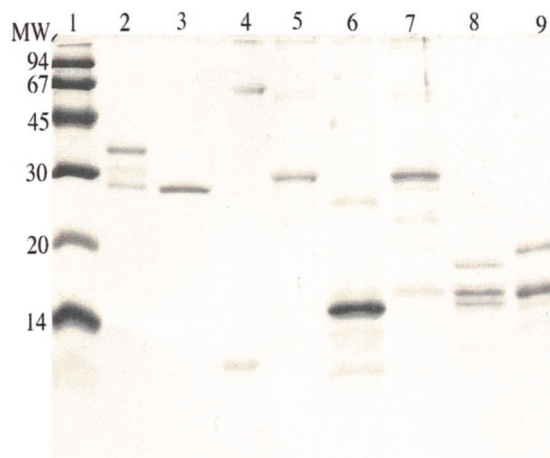


Figure 3. SDS-PAGE analysis of recombinant LTP from *Hevea*. Proteins were separated on a 15% gel under reducing conditions and stained with Coomassie blue. Lane 1, molecular weight markers; lane 2, GST/LTP; lane 3, GST vector control; lane 4, thrombin digest of GST/LTP; lane 5, pET32/LTP expressed in pLysS; lane 6, thrombin digest of pET32/LTP; lane 7, pET32/LTP expressed in TrxB; lane 8, enterokinase digest of pET32/LTP; lane 9, pET32a vehicle control.

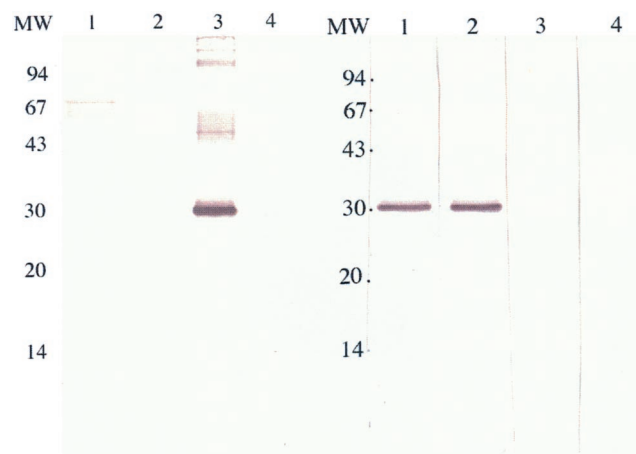


Figure 4. Western analysis of rLTP using cross-reacting rabbit antibodies. Panel A represents rabbit anti-apple LTP reactivity to *Hevea* LTP. Lane 1, ammoniated latex protein; lane 2, non-ammoniated latex protein; lane 3, pET32/LTP; lane 4, pET32a vehicle control. Panel B represents rabbit anti-apple LTP staining of *Hevea* LTP. pET32a/LTP (1 μ g/lane in reducing sample buffer) was loaded in all lanes with the antisera inhibited as follows: lane 1, no inhibition; lane 2, inhibited with pET32a control (20 μ g/mL); lane 3, inhibited with pET32a/LTP (20 μ g/mL); lane 4, inhibited with LTP cut from GST/LTP (10 μ g/mL).

sera, weak reactivity, presumably background, was noted to pET32a control protein containing thioredoxin and the affinity tag regions. Thioredoxins are known allergens, thus this weak IgE binding could be a cross-reactivity or co-sensitization to thioredoxin. The allergen nomenclature committee of the International Union of Immunological Societies approved the designation of Hev b 12 for the LTP from *Hevea*.

It has been noted that the disulfide bonds are important for IgE reactivity to LTPs.²³ Two patients allergic to LTP from fruit (PF227, PF194) recognized *Hevea* LTP under non-reducing conditions, with only weak reactivity to the reduced protein (Fig 6). These patients recognized the pET32/LTP produced in both pLysS and TrxB-negative bacterial strains indicating that the disulfide bonds important for secondary structure formed in both strains (data not shown). Four LTP positive latex-allergic sera were tested against the reduced and non-reduced rLTP and found to only react to rLTP under reducing conditions (Fig 6). This suggests the patients were



Figure 5. Immunoblot of sera from 18 latex-allergic patients against pET32a/LTP. The 30-kDa fusion protein of pET32a/LTP (1 μ g/lane) was separated by SDS-PAGE on 15% gels under reducing conditions and transferred to nitrocellulose. Individual strips were probed with a 1/10 dilution of serum from individual latex-allergic patients. Molecular weight markers stained with Ponceau S (Sigma, St. Louis, MO) are shown in the left lane.

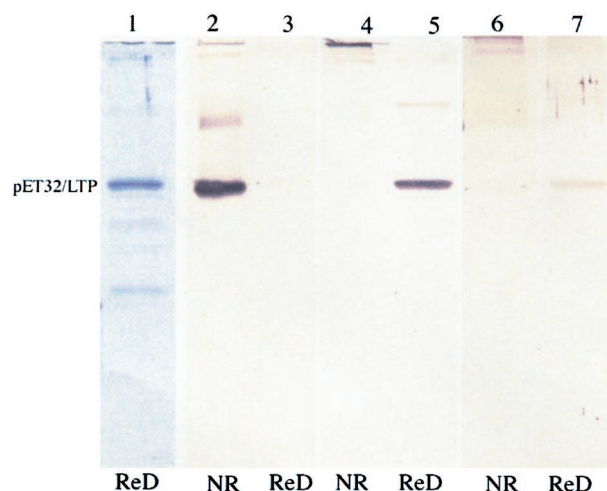


Figure 6. Comparison of immunoblot reactivity to pET32a/LTP electrophoresed under reducing and non-reducing conditions. Lane 1 is SDS-PAGE of pET32/LTP. Lanes 2 (non-reduced) and 3 (reduced) were reacted with IgE from a patient with anti-fruit LTP reactivity. Lanes 4 (non-reduced) and 5 (reduced) were reacted with sera from a latex-allergic patient reactive with Hev b 12 (1 of 4 shown). Lanes 6 (non-reduced) and 7 (reduced) were reacted with rabbit anti-non-ammoniated latex protein sera.

sensitized to *Hevea* LTP, rather than cross-reacting as a result of sensitization to LTP from foods, as the natural rubber protein found on latex products is predominantly found in a denatured form. Supporting this idea, sera from rabbits immunized with latex proteins also reacted to the reduced and not the non-reduced *Hevea* LTP (Fig 6).

DISCUSSION

Latex-allergic individuals commonly exhibit clinical cross-reactions to fruits, vegetables, and other plant materials.^{14,24–26} Particularly common is cross-reactivity to a small number of fruits including banana, avocado, and kiwi.¹³ This cross-reactivity has largely been attributed to hevein or hevein-like domains (Hev b 6.02) of class I chitinase proteins in these foods.^{27,28} Class I chitinases belong to the PR-3 family of pathogenesis-related proteins.¹ Cross-reactivity to other fruits including apple, cherry, and peach is also reported for latex-allergic individuals, but with less frequency.^{14,29} LTPs are

classified as pan-allergens of the PR14 family¹ and a major allergen in Rosaceae allergy when pollinosis is not involved.⁴ The screening of cDNA expression libraries with IgE from patients has been a useful tool for identification of allergens. In this study we used molecular techniques to target specifically a potentially cross-reactive allergen (LTP). We sought to determine whether LTP was present in *Hevea* and whether it was an allergen. Here we present the first description and initial characterization of the LTP from *Hevea* latex.

We identified a 662-bp cDNA with a 351-bp open reading frame that encodes for a 116-amino acid protein, with a signal peptide of 24 amino acids. The protein sequence was found to have significant homology to the family of nonspecific LTPs. One potential glycosylation site was noted, but this site was not conserved in other LTPs, and LTPs in general do not appear to be glycoproteins. We expressed the protein as a mature LTP with a predicted molecular weight of 9.3 kDa and pI of 10.8. The protein was expressed in two different *E. coli* expression systems (GST and pET32) to allow flexibility, because of potential bacterial toxic effects of LTP and to enhance the formation of secondary structures. rLTP from latex did not appear bactericidal in that we were able to express the fusion protein in both GST and pET32 expression systems. rLTP appeared to acquire proper secondary structure formation in both the TrxB-negative and the pLysS bacterial strains because IgE from fruit LTP-allergic patient that only reacted to LTP under non-reducing conditions recognized the pET32/LTP produced in both strains.

Immunoblots with patient sera demonstrate *Hevea* LTP IgE reactivity in sera from 24% of latex-allergic individuals. Interestingly, latex patients' IgE reacted to LTP under reducing conditions but not under non-reducing conditions. The designation of *Hevea* LTP as Hev b 12 was approved by the International Union of Immunological Societies. The prevalence of IgE reactivity to Hev b 12 in a larger population of latex-allergic individuals remains to be determined. Based on the low prevalence of clinical cross-reactivity to fruits such as apple and peach,²⁹ it is probable that Hev b 12 is not a major latex allergen; however, it is potentially important as a cross-reactive allergen for patients with food cross-reactivity.

Hevea LTP appears to be highly cross-reactive with LTPs from other plants. IgE from two patients with reactivity to fruit LTP cross-reacted with *Hevea* LTP. Antibodies from rabbits immunized to LTPs from apple, peach, and carrot also recognized *Hevea* LTP and could be inhibited with the soluble LTP cut from the GST fusion protein. Two of the 3 cross-reacting rabbit sera (apple and peach) only weakly recognized LTP in immunoblots when it was cut from its fusion partner, suggesting that there may be conformational changes in the protein during the binding to nitrocellulose. Alternatively, blotting small basic proteins such as LTP is difficult and may result in a reduced number of epitopes exposed and available for antibody recognition. A similar observation, where the protein needs to remain as a fusion protein to detect IgE reactivity, has been made for Hev b 5.³⁰

The question of whether LTP protein is expressed in latex or found on manufactured latex products proved to be elusive. Using cross-reacting rabbit antisera, we found reactivity to a protein from latex, but we were unable to inhibit that reactivity with rLTP from *Hevea*. Patients reacting to fruit LTP had IgE reactivity to several proteins not recognized by control sera, but again we were unable to inhibit this reactivity in Western blots (data not shown). In contrast, latex-allergic patients and antisera from rabbits immunized with latex proteins recognized rLTP from latex. So, although we were able to identify the cDNA for LTP in *Hevea* latex, we could not conclusively demonstrate that the protein was expressed in latex.

There was an interesting difference in the patient reactivity to LTP. Fruit LTP allergic patient sera only reacted to *Hevea* LTP when the protein was in the non-reduced form, as has been demonstrated for *Parietaria* LTP.²³ In contrast, latex-allergic sera LTP IgE binding activity was stronger toward the reduced form of the fusion protein. This could be explained by the fact that the sensitizing protein remaining on latex products is largely denatured, perhaps exposing cryptic epitopes. Without further study, it is not known whether the patients reacted to *Hevea* LTP because of sensitization to *Hevea* LTP on latex products or because of cross-reactivity attributable to a primary sensitization to LTP in fruits.

CONCLUSION

The current diagnostic tests for latex are less than optimal, demonstrating approximately 75% sensitivity and 95% specificity.³¹ Recent studies have begun to explore the possibility of using a select group of recombinant proteins to improve these diagnostic tests. Yip et al¹⁹ skin-tested 31 latex-allergic patients with Hev b 2, 3, 5, 6, 7, and 8 and found that Hev b 5, 6, and 7 could be used to detect up to 93% of the latex allergy. Similarly, skin tests with Hev b 1 to 7, in 60 patients found that 8% were skin test-negative to the all of the purified proteins.³² As 7 to 8% of patients do not react to any of the known *Hevea* allergens, there may be unrecognized allergens or epitopes that could account for the discrepancy. Studies are underway to determine whether Hev b 12 is one of the missing allergens. Preliminary studies indicate that the lack of sensitivity of these tests may be a result of missing allergenic epitopes.³³ Identification of the missing allergens or epitopes has the potential to further improve the efficiency of the diagnostic tests.

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