

Cloning of the Patatin-Like Latex Allergen Hev b 7, Its Expression in the Yeast *Pichia pastoris* and Its Immunological Characterization

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Key Words

Hevea brasiliensis · Latex · Allergy · Hev b 7 ·
Recombinant allergen · Patatins

Abstract

The 43-kD latex allergen Hev b 7 was purified from the latex of *Hevea brasiliensis* and identified by N-terminal and internal peptide sequences as highly homologous to patatins. Patatins are storage proteins encoded by a multigene family found in plants such as potato and tomato. We have obtained a cDNA clone coding for a cytoplasmic form of Hev b 7. The recombinant protein was expressed in the methylotrophic yeast *Pichia pastoris* at 10 mg/l culture supernatant. Both natural Hev b 7 and rHev b 7 were recognized by IgE in 11% of the latex-allergic patients. rHev b 7 inhibited binding to its counterpart in natural rubber latex extracts. Purified rHev b 7 used at concentrations of 10 µg/ml in skin prick tests produced wheal-and-flare reactions of sizes equal to those produced by nHev b 7. Furthermore, we were able to show that rHev b 7 possessed esterase activity. A plant expression system for the production of larger quantities of recombinant latex allergens as an alternative to the preparation from *H. brasiliensis* sap is discussed.

Natural rubber latex is widely used in the manufacturing of medical devices and in a variety of everyday articles. Latex is the milky sap produced by the laticiferous cells of the tropical rubber tree *Hevea brasiliensis*. Immunoglobulin E mediated allergy to natural rubber latex is widely acknowledged as a major occupational problem. Latex allergy is frequently seen in health care workers, rubber industry workers and subjects undergoing multiple surgical procedures in early infancy. Symptoms manifest as contact urticaria, rhinoconjunctivitis or mucosal swelling. Systemic reactions consist of generalized urticaria, asthma and anaphylactic shock. *Hevea* latex contains roughly ten important allergens among several other allergens and hundreds of other abundant proteins and organic compounds [1, 2]. Allergen contents and relative amounts of individual allergens in total latex extracts are subject to variation. To date, no purified or recombinant latex allergens are commercially available for diagnosis.

A 43-kD latex allergen designated Hev b 7 was identified by N-terminal and internal peptide sequencing as highly similar to patatins which are storage proteins encoded by a multigene family found in plants such as potato and tomato [3]. We synthesized double-stranded cDNA prepared from 5 µg of poly(A)⁺ *Hevea* latex mRNA and ligated it into the λ ZAP II vector. We obtained a cDNA clone coding for a cytoplasmic form of Hev b 7. The hybridization probe comprised a conserved region of *Solanum tuberosum* and *Nicotiana tabacum* patatins to which four natural (n) Hev b 7

derived peptides could be aligned [4]. The Hev b 7 cDNA has an open reading frame of 1,164 bp which encodes a polypeptide of 388 amino acid residues with a predicted molecular weight of 42,871 Da and a calculated pI of 5.0. The deduced amino acid sequence lacks an N-terminal leader peptide. Despite the fact that the glycosylation sites in the Hev b 7 sequences were functional, nHev b 7 was not glycosylated. Hev b 7 shares substantial sequence similarities with patatins from *S. tuberosum* and *N. tabacum* (43% identity with *N. tabacum* patatin and 39–42% with different patatins from *S. tuberosum*). Peptides derived from the purified nHev b 7 could be aligned to the deduced amino acid sequence of Hev b 7.

The recombinant protein could be expressed in the methylotrophic yeast *Pichia pastoris* at an expression level of 10 mg recombinant (r) Hev b 7 per liter culture. rHev b 7 bound IgE of 4/36 (11%) latex-allergic patients. The IgE binding to nHev b 7 present in *Hevea* latex could be inhibited by preincubating sera with 10 µg rHev b 7. rHev b 7 displayed esterase activity, indicating that it was correctly folded and equivalent to its natural counterpart [5]. Purified rHev b 7 used at concentrations of 10 µg/ml in preliminary skin prick testing produced wheal-and-flare reactions of sizes equal to those produced by nHev b 7. Hev b 7 is only a minor component of *Hevea* latex, and preparing sufficient amounts of purified nHev b 7 is difficult and time-consuming. rHev b 7 represents an economic alternative to the preparation of nHev b 7 from *H. brasiliensis* sap.

Latex allergy can have serious and fatal outcomes. Avoidance of latex products or exposure to latex allergens can prevent serious allergic reactions to latex proteins. Given the ubiquity of latex in household and medical devices, complete avoidance may be an impossible task. As preventive measures one can envision the use of alternative non-la-

tex products and the reduction of the allergen content of latex medical devices. However, these approaches will be costly, and one cannot expect their total or immediate application.

Treatment strategies are possible which may allow latex allergics to remain in their current careers such as health care. One such is immunotherapy which has been used extensively as a specific treatment of type I allergy. Immunotherapy of latex allergy is not possible with currently available crude preparations. Pharmaceutical-grade recombinant allergens with immunological properties identical to the natural allergens would be the reagents of choice for specific immunotherapy. Another strategy was applied at the Thomas Jefferson University in Philadelphia where health care workers with severe latex allergy symptoms underwent the first oral latex desensitization scheme [6].

Oral desensitization schemes require large amounts of pure allergens to be administered. Plant proteins are ideally expressed in plants, rather than in *Escherichia coli* or yeast. Expression of heterologous proteins in plants also meets the need for scalable and cost-effective production for such an application. Tobacco mosaic virus is one of the more useful gene transfer vectors for whole plants [7]. Tobacco mosaic virus mediated transient expression of latex allergens would be a natural next step in the application of this convenient system to produce large quantities of recombinant proteins for a variety of uses from diagnosis to specific immunotherapy.

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