

Pt2L4 Protein, a Homologue to Hev b 5 from Rubber Tree, May Not Be Responsible for the Cross-Reactions to Cassava Show by People Allergic to Latex

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Abstract: Pt2L4 is a protein from cassava homologue to Hevb5, a principal allergen from latex. Here we aimed to elucidate immunological relationships between these proteins. Our results revealed that epitopes found in Hev b 5 are not entirely conserved in Pt2L4 which is not recognized by IgE from patients allergic to Hev b 5.

Keywords: Allergenicity, Cassava, Glutamic acid-rich protein, Latex-fruit syndrome, Storage root formation.

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INTRODUCTION

Pt2L4 is a glutamic acid-rich protein related to secondary growth and storage root formation in cassava (*Manihot esculenta* Crantz) [1, 2] that is 42% identical to Hev b 5 protein from rubber tree [3], a major allergenic agent with a reported prevalence of specific serum IgE of 52% to 92% among allergic patients to latex [4, 5]. Less than 1% of the general population is allergic to latex; however the prevalence of latex allergy is higher in people who wear latex gloves at work as sanitary workers (17%) and in people who have suffered many surgical operations, like *Spina bifida* children (50%) [6]. About 21 and 58% of latex-allergic patients show allergic responses to banana, papaya, avocado and kiwi fruits, also called latex fruit syndrome [7]. Allergy to cassava attributed to cross-reactivity with latex has been reported [8-10].

The molecular basis of allergenic cross-reactivity is the occurrence of homologous proteins (or protein domains) in both the primary sensitizer and the cross-reactive allergenic sources. Homologous proteins share different degrees of sequence identity, similar 3D-structures and common epitopes recognized by IgE antibodies [11]. Homologues to Hev b 5 protein contain a high glutamic acid and alanine content, pI value in the range of 3.6-3.8 and molecular mass around 18 kDa and they have been found in kiwi fruit [4, 5], sugar beet [12] and cassava [3].

Cassava storage roots are a staple food in Africa, South America and Asia. People allergic to cassava have been reported [8-10]; therefore, the identification of cassava proteins with allergenic properties is highly relevant. In this work we aimed to evaluate immunological relationships between Pt2L4 and Hev b 5 proteins. Blots with Pt2L4 protein were tested for cross-reactivity against polyclonal antibodies raised against latex and Hev b 5 protein and human IgE from hypersensitive allergic patients to Hev b 5. A comparative sequence analysis was conducted in order to search the Pt2L4 protein for IgE epitopes identified in Hev b 5 by Beezhold *et al.* [13].

MATERIAL AND METHODS

Plant Material

Plants of the commercial cultivar IAC 12-829 were propagated by stem cuttings and planted in field plots of EMBRAPA – Genetic Resources and Biotechnology and grown for 10 months. Storage roots were harvested and roots of uniform 5 cm diameter were sampled to dissect tissue layers as previously described [2]. Dissected tissue from layer 4 was used in Pt2L4 extraction as previously described [14].

Antibodies Raised Against Latex and Hev b 5 Proteins

Human sera with anti-Hev b 5 IgE, rabbit anti-latex and anti-Hev b 5 IgG were obtained as previously reported by Beezhold *et al.* [13, 15]. The Hev b 5 reactive IgE serum was pooled from 6 latex allergic patients with positive skin tests to recombinant *Hev b 5* at 1 ng/ml or less.

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Western Blot Analysis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblots were performed as described by Beezhold *et al.* [13].

Sequence Analysis

Sequences of Pt2L4 from cassava (AAM55492) and Hev b 5 from rubber-tree (Q39967) were imported from Swis-sprotein Bank and aligned using Clustal program.

RESULTS AND DISCUSSION

Patients who are allergic to latex may exhibit cross-hypersensitivity with foods, including cassava which belongs to the *Euphorbiaceae* family and it is often used as a staple food in Africa, Asia and Latin America. Cross-reactivity between different foods occurs due allergenic proteins showing common IgE epitopes [11].

In this work we used IgG antibodies raised against latex and Hev b 5 protein and IgE from patients allergic to Hev b 5

in order to identify cross-reactivity between Pt2L4 protein from cassava and a major latex allergen. These proteins have unusually high levels of glutamic acid, alanine and proline residues and migrate at 32-36 kDa in SDS-PAGE although they have a predicted molecular weight of 16-18.0 kDa [2, 4, 5]. Differences between predicted and apparent molecular mass is often seen with acidic proteins and has been observed by others [16, 17].

Studies revealed that in Western blot experiments Hev b 5-specific antibodies recognize a broad range of protein in the 36- to 100-kD [14, 18] and a similar pattern was also detected in Pt2L4 sample (Fig. 1). Our results show that Pt2L4 protein is recognized by IgG antibodies raised against latex and Hev b 5 protein, but not by human IgE from hyper-sensitive patients allergic to Hev b 5.

The recognition of Hev b5 by sera pooled from Hev b 5-allergic patients has been attributed to 11 different IgE epitopes [13] which are mostly located in regions of alignment gaps or non-homology with the Pt2L4 protein (Fig. 2). The results obtained here indicate that despite these two proteins

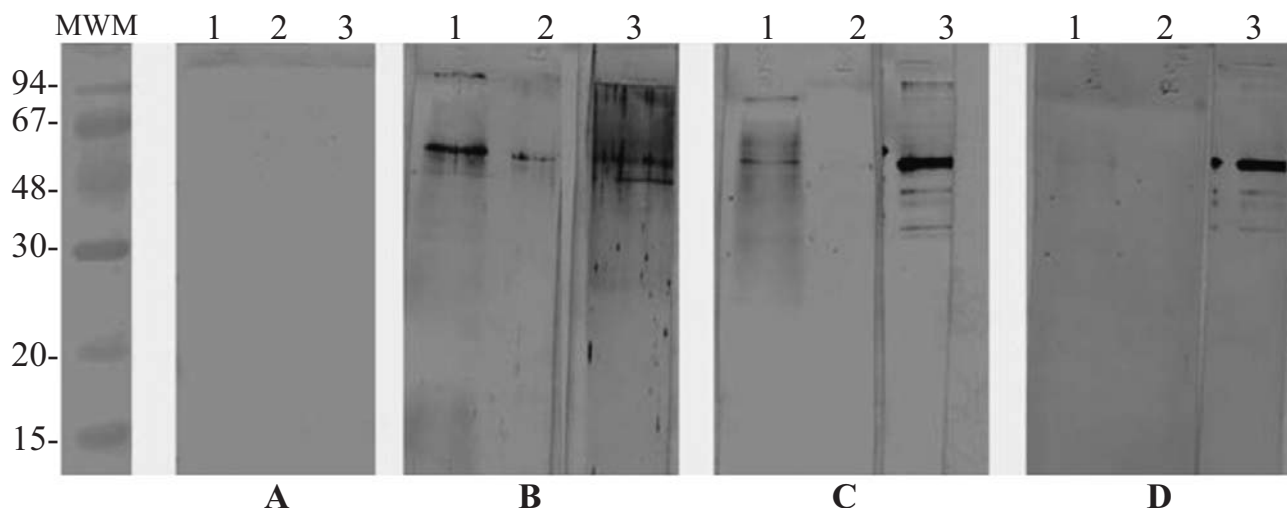


Figure 1. Western blot analysis of Pt2L4 and Hev b 5 proteins probed with different antibodies: A- Rabbit pre immune serum, B- Rabbit IgG raised against latex, C-Rabbit IgG raised against Hev b 5 and D- Human IgE from hypersensitive patients allergic to Hev b 5. Proteins were separated in SDS- PAGE, blotted on a nitrocellulose membrane and probed with antibodies. MWM: molecular weight maker (kDa), 1: Pt2L4 protein, 2: Bovine Serum Albumin (as a control) and 3: Hev b5 protein.

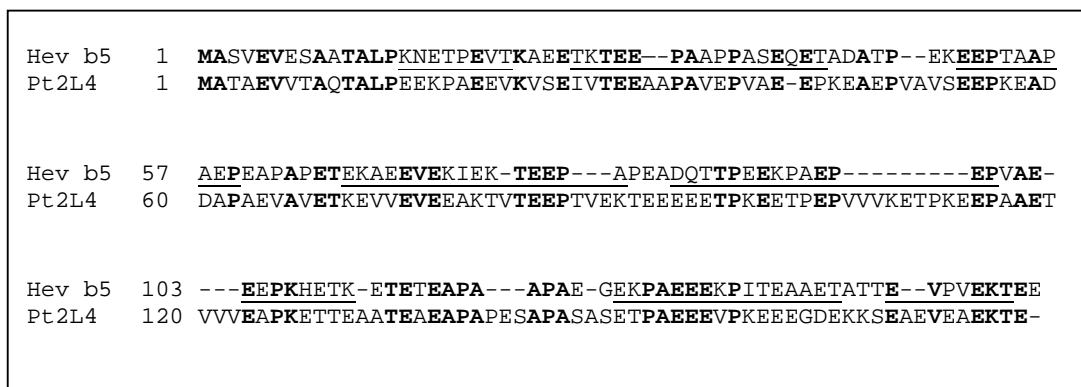


Figure 2. Sequence alignment between Pt2L4 from cassava (AAM55492) and Hev b 5 from rubber-tree (Q39967) using Clustal program. Dashes are sequence gaps. IgE epitopes identified in Hev b 5 by Beezhold *et al.* [13] are underlined.

have antigenic similarities, the Pt2L4 may not be responsible for the allergic cross-reactions to cassava showed by people allergic to latex. Other proteins possibly related to this cross-reactivity can include a 42-44 kDa protein similar to Hev b 7 or patatin-like protein [9] and a homologue to prohevein (Hev b 6) [10].

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