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SHORT COMMUNICATION
Hev b 11, a Peculiar Class I Chitinase?

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The recently identified rubber allergen Hev b 11, which is a class I chitinase, may be a cytosolic (C-serum) protein. This is a rather unique feature, as all other known plant class I chitinases are vacuolar proteins.

Key words: allergen; Hev b 11; *Hevea brasiliensis*; chitinase; hevein(-like) domain

The study of natural rubber latex is not only valuable for its commercial applications, but also for fundamental contributions in the fields of life and material sciences. Interesting observations are made in several disciplines. A rather recent field of rubber latex studies is the investigation of proteins which cause latex allergy with much importance for human health[1]. These studies, generally published in the medical literature, may also lead to interesting conclusions on the physiology and biochemistry of rubber latex in a broader sense. However, this is not always recognised in these publications.

Several years ago, a novel rubber latex allergen was discovered, first by microsequencing of a protein identified by immunoblotting[2] and later by cloning and sequencing of the cDNA clone encoding this allergen[3,4]. The protein was found to be a class I chitinase, has an N-terminal hevein-like domain, a molecular mass of 33 kDa and received the designation Hev b 11.

In a classification of glycosyl hydrolases[5], plant chitinases belong either to family 18 or family 19. There is no sequence homology[6] or structural similarity[7] between members of the two families. In another classification of plant chitinases, family 18 enzymes belong to class III, and the family 19 ones to classes I, II and IV[8]. Generally class I chitinases are basic proteins with a hevein-like domain preceding the catalytic domain, while class II are more acidic and lack a hevein-like domain. Almost all chitinases from families 18 or 19 are extracellular or vacuolar proteins, synthesised with an N-terminal signal peptide which is cleaved off in the mature protein[8].

Although many studies have been performed on rubber latex proteins, a class I chitinase had never been observed before. The only other chitinase known in rubber latex was hevamine, a class III or family 18 chitinase from the lutoid body or bottom fraction (B-serum). This protein is already known since the pioneering studies by Tata *et al*.[9] Three variants of hevamine with a mass of about 30 kDa could be identified in the bottom fraction of rubber latex, but a thorough investigation of B-serum preparations did not give any indication of the presence of a representative family 19

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chitinase[10]. Hevamine is not recognised as a major rubber latex allergen[1].

What may have been the reason that the class I chitinase was not identified earlier in rubber latex preparations? This may be explained by its location in the cytoplasm (C-serum) of rubber latex, a fraction less intensively investigated than the B-serum (Iutoid body fraction). O'Riordain *et al*.[3] and Rihs *et al*.[4] present almost identical sequences of the mature class I chitinase (Hev b 11) of 295 amino acid residues, but do not mention investigations on the cellular location of the protein. The given open reading frames of 888 b.p.[3] or 885 b.p.[4] are in agreement with this polypeptide length (888 b.p. includes the stop codon), but in neither paper is it mentioned that the N-terminal residue is preceded by a cleaved off initiator amino acid residue in the message or by a signal peptide sequence (in which case the presented open reading frame length would not be correct). The absence of a signal peptide sequence in Hev b 11 is not yet proven, but a location of the mature protein in the cytosol (C-serum) is suggested by results presented in *Figure 5* in the paper by O'Riordain *et al*.[3], which shows binding of IgE from patients allergic to Hev b 11 by extracts of B- and C-serum. In C-serum, there is the expected reaction of Hev b 11 with a molecular mass of 33 kDa, while in B-serum only the hevein precursor, a protein with a molecular mass of 21 kDa reacts, which can be explained by the presence of shared epitopes on the hevein (-like) domain of Hev b 11 and the hevein precursor, but no reaction of a 33 kDa protein.

A location of Hev b 11 in the cytosol may also explain that its N-terminal hevein-like domain is not cleaved off. Hevein (5 kDa) is formed by a still unknown proteolytic process, probably directly after translation of the protein at membranes involved in transport of vacuolar proteins to the lutoid bodies[11]. But a relatively small amount of the hevein precursor can still be identified in lutoid bodies. A similar processing occurs in the production of stinging nettle agglutinin, in which a tandem of two linked hevein-like domains are cleaved off from a precursor[12]. It is possible that the sequence of the hinge peptide between the hevein and the chitinase domains of Hev b 11 is not susceptible to the proteolytic enzyme(s) cleaving the hevein precursor. However, it is more likely that a different cellular location prevents this processing.

It is interesting that the presence of a class I chitinase in rubber latex has been demonstrated in earlier studies, but that the authors of these publications were not aware of this fact, as they probably assumed that hevamine, the class III chitinase, was the protein identified by them. Gidrol *et al*.[13] used a class I chitinase probe from *Phaseolus vulgaris*[14] to demonstrate that etephon treatment increases the expression of chitinase in rubber clone WAR4, but less in clone GT1. Martin[15] demonstrated the presence of chitinases with an antibody against the chitinase from *Phaseolus vulgaris*. However, cross-reacting proteins were only found in B-serum and not in C-serum, which is not in agreement with current expectation.

A cytosolic location of a class I chitinase is unique. In the cDNA sequences of all other class I plant chitinases in the database the open reading frame always consists of a chitinase domain, preceded by a hevein-like domain and a signal peptide sequence. But as the lutoid contents mix after bursting with the cytosol, the final fate of latex proteins is less dependent on their original location.

This short review on this new chitinase shows that much knowledge is still lacking. It may be suggested to start additional investigations, like performing a 5'RACE to identify the 5' -start of the Hev b 11 message. But it is more important to isolate mature class I chiti-

nase from rubber latex, and to study its cellular location, enzymic properties and potential antifungal properties (in synergy with other antifungal proteins in rubber latex). In general, more studies on rubber latex proteins will be useful. Possibly there are economically useful applications of these natural products and their removal from the watery effluents of natural rubber plants will not only be advantageous for the environment. There are similar examples in other agricultural industries of proteins originally polluting the environment, which are now valuable, like whey proteins produced during the production of cheese from milk.

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