

Editorial

Thaumatococcus-like proteins – a new family of pollen and fruit allergens

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Pathogenesis-related proteins (PRs) are now widely regarded as a rich source of allergens (1, 2). The PRs are defined as proteins that are encoded by the plant genome and induced specifically in response to infections by pathogens such as fungi, bacteria, or viruses, or by adverse environmental factors. Pathogenesis-related proteins do not constitute a superfamily of proteins but represent a collection of unrelated protein families which function as part of the plant defence system. Today, PRs are classified into 14 families (3). Many important plant food allergens are homologues to proteins that are members of PR families (4, 5).

The family 5 of PRs comprises unique proteins with diverse functions. Because of the sequence homologies between PR-5 proteins and thaumatococcus, an intensely sweet tasting protein isolated from the fruits of the West African rain forest shrub *Thaumatococcus daniellii*, members of this family of proteins are referred to as thaumatococcus-like proteins (TLPs). TLPs can be classified into three groups, (i) those produced in response to pathogen infection, (ii) those produced in response to osmotic stress, also called osmotins, and (iii) antifungal proteins present in cereal seeds. The TLPs are generally resistant to proteases and pH- or heat-induced denaturation. This is likely due to the presence of 16 conserved cysteines that form eight disulfide bridges. The crystal structure of three PR-5 type proteins, PR-5d from tobacco (6), zeamatin from *Z. mays* (7), and thaumatococcus from *T. daniellii* (8) that are known to date illustrate the high conformational similarity between these proteins.

Several allergenic TLPs from fruits have been described. Mal d 2 is a 23 kDa allergenic TLP of apple fruits that induces IgE-mediated symptoms in apple allergic

individuals. This apple protein whose amino-terminal sequence shares about 50% identity with PR-5 family members was the first TLP described as an allergen (9). The cDNA sequence of Mal d 2 was expressed in *Nicotiana benthamiana* plants using a recombinant tobacco mosaic viral vector (10). Purified recombinant Mal d 2 displayed the ability to bind IgE from apple-allergic individuals equivalent to natural Mal d 2. In addition, the recombinant thaumatococcus-like Mal d 2 exhibited antifungal activity against *Fusarium oxysporum* and *Penicillium expansum*, indicating a function in plant defense against fungal pathogens. In sweet cherry (*Prunus avium*), a 23 kDa thaumatococcus-like protein was identified as a major allergen, designated Pru av 2 and its cDNA cloned (11). The N-terminal sequence of a 23 kDa bell pepper allergen was determined to be identical to the corresponding portion of the osmotin-like protein P23 from tomatoes (12). The complete coding sequence (EMBL AJ297410) of this bell pepper fruit allergen designated Cap a 1 was obtained (13). An allergenic 24 kDa TLP from kiwi fruit was shown to be glycosylated and to possess antifungal activity against *Saccharomyces carlsbergensis* and *Candida albicans* (14). Very recently, a 24 kDa TLP was identified as a minor allergen of grape with an amino acid sequence highly similar to Mal d 2 and Pru av 2 (15). This brings the number of allergenic plant food TLPs up to five. So far, only one allergenic pollen TLP had been identified, the mountain cedar (*Juniperus ashei*) pollen allergen Jun a 3 which was also the first allergenic TLP whose expression in the cytoplasm of *Escherichia coli* was published (16).

In this issue of Allergy, Cortegano et al. (17) describe the PCR cloning and expression of the *Cupressus*

arizonica pollen allergen Cup a 3. Cup a 3 is the second allergenic pollen TLP that has been discovered so far. *C. arizonica* belongs to the family Cupressaceae as does *J. ashei*. *C. arizonica* pollen constitutes an important allergen source in Spain especially from January to April, whereas *J. ashei* pollen causes severe seasonal allergic disease in broad areas of the southern central United States and northern Mexico (16). The Jun a 3 amino acid sequence contained a single potential N-glycosylation site and the natural purified protein bound to the lectin concanavalin A. In contrast, the Cup a 3 sequence does not contain any N-glycosylation sites. Among the plant food allergens, Mal d 2 and Pru av 2 possess potential N-glycosylation sites (10, 11), and the allergenic kiwi TLP has been shown to be glycosylated by its ability to bind to concanavalin A (14). For the past few years, there has been an ongoing discussion whether the carbohydrate moiety of glycoallergens contributes to their immunoglobulin (Ig)E-binding capacity and ability to elicit IgE-mediated allergic symptoms. The various glycosylated and nonglycosylated allergenic TLPs may be used to establish a model system to find an answer to this debate.

Today, recombinant allergens are the diagnostic and therapeutic molecules of choice. In this context, TLPs offer two challenges. First, their conformation and probably the majority of their IgE-binding capability depends on the correct formation of the eight conserved disulfide bonds. Secondly, the presence of plant-specific carbohydrates may play a minor but important role in the molecule's allergenicity. When expressing recombinant thaumatin or allergenic TLPs in bacteria, yeast, insect cells, and fungi several problems have been reported such as incorrect processing, incorrect three-dimensional folding and insufficient yields of the pure recombinant protein

(18–20). Therefore, the expression system of choice may be a plant-based one. Although it is time-consuming to establish, the chances that the resulting recombinant protein will be properly folded and glycosylated are greater than in bacterial or yeast expression systems. Mal d 2 has been expressed in *N. benthamiana* plants and the recombinant allergen displayed the ability to bind IgE from apple allergic individuals equivalent to natural Mal d 2 (10). However, only continuing research will propose alternative but not mutually exclusive ways to produce pure allergenic TLPs.

Cortegano's paper also addresses the influence of environmental pollution on the expression of Cup a 3 in pollen. It had been previously shown that pollen of different trees of *J. ashei* in different years contained variable levels of the allergenic TLP Jun a 3 (16). These authors speculated that environmental conditions might be the cause of the altered expression levels thus influencing the allergenicity of the pollen. Cortegano and coworkers provide the reader with data describing the effect of environmental pollution on the expression of Cup a 3. It is of interest to note that the expression of Cup a 3 was only detectable in pollen from trees growing in an industrial area near a highway with heavy traffic but not in pollen collected from trees planted in a garden in a residential area. In contrast, it was found that air pollutants did not have any effect on expression levels of Bet v 1, the major birch pollen allergen and a homologue to PR-10 family members (21). An explanation could be that Bet v 1 fulfils a function that is not exclusively connected to plant defence (22).

In conclusion, one can safely say that this study by Cortegano et al. and the earlier studies mentioned above have firmly established TLPs as an allergen family present in pollen and plant foods.

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