ABSTRACT OF THE THESIS

Title: Biochemical and immunological characterization of the major allergen from sapodilla fruit (*Manilkara zapota*)

Allergy or hypersensitivity affects sensitized individuals, which results from inappropriate immune responses, often to common antigens such as plant pollens, foods, dust, animal dander, mold spores, insect products, metals, drugs, etc. Food allergies are mainly IgE-mediated type I hypersensitivity reactions, wherein ingested food proteins elicit allergic symptoms in predisposed individuals. It is estimated that 2–4% adults and 6–8% children worldwide suffer from one or the other kind of food allergy. Clinical outcome of food allergy vary from the mild oral allergy syndrome (OAS), erythematous rashes, vomiting, diarrhea to fatal systemic anaphylaxis. Although an individual can be allergic to any food (plant or animal source), the following 8 foods have been found to account for ~90% of all food-allergic reactions: milk, eggs, fish, crustaceans, tree nuts, peanuts, wheat and soybean. Apart from these 8 foods, other foods including cereals and grains, legumes, fruits and vegetables also contribute toward food allergy cases.

Several common fruits (apple, apricot, banana, cherry, date, grape, kiwi, mango, melon, orange, peach, pear, pineapple, pomegranate and strawberry) have been studied with respect to their allergenicity. Sapodilla fruits (*Manilkara zapota* syn. *Achras zapota*), also known as sapota, chikoo in India, are round, ovoid or ellipsoid, are cultivated in the tropical parts of the world with India being the largest grower. Allergic reactions to the ingestion of sapodilla fruit are rare; only 3 cases of sapodilla allergy displaying OAS have been described earlier, wherein a ~21 kDa protein was identified as the causative allergen. The present investigation focuses on biochemical and immunological characterization of the 21 kDa major allergen from sapodilla, and the study was carried out by using various *in vitro, in vivo* and *in silico* methods commonly used in allergology. The introductory aspects of food allergy and description of allergy to different fruits are covered in Chapter 1.
Chapter 2 describes the biochemical purification, identification and characterization of the 21 kDa major allergenic protein from sapodilla fruit pulp as an acidic thaumatin-like protein (TLP). Sapodilla-allergic cases were investigated by skin prick test, allergen-specific ELISA and IgE-immunoblot. The allergenic acidic TLP, purified using SP-Sepharose chromatography was found to have a pI of ~4.6 and due to its high disulfide content shows variation in mobility upon electrophoresis under reducing and non-reducing conditions. Purified acidic TLP has been obtained in a yield of ~6.8 mg per kg sapodilla pulp. Acidic TLP showed weak β-1,3-glucanase activity using laminarin (β-1,3/1,6-glucan) as substrate. Other proteins in sapodilla pulp were also identified which included the 21 kDa basic TLP, 28 kDa basic chitinase and 37 kDa basic β-1,3-glucanase.

The gene sequence encoding the sapodilla acidic TLP was determined by genomic cloning, and is presented in Chapter 3. The N-terminal sequence of the acidic TLP and conserved regions among other plant TLPs were used to design two sets of nested degenerate primers. A partial acidic TLP gene (devoid of nucleotides coding for the signal peptide and 7 residues from the N-terminus; NCBI Genbank JN624813.1), found to be intronless was obtained which codes for 200 amino acid residues. The deduced composite sequence of sapodilla acidic TLP has a theoretical molecular mass of 21922.4 and a pI of 4.44. NCBI Conserved Domain Database search revealed that the sapodilla acidic TLP allergen has domains which fit into the superfamily glycoside hydrolase family 64 (GH64) and thaumatin-like proteins (TLP).

The structural and allergenicity features of sapodilla acidic TLP as investigated by in silico methods, are described in Chapter 4. The allergenicity prediction was performed using FARRP, Allermatch and Evaller web tools. A homology model of the protein was generated using banana TLP template (1Z3Q) by HHPRED-MODELLER. B-cell linear epitope prediction was performed using BCpreds and BepiPred. IgE epitope prediction as performed using AlgPred indicated the presence of 2 epitopes and a comprehensive analysis of all allergenic TLPs displayed up to 3 additional epitopes on other TLPs. The
secondary structural elements of TLPs vary markedly in regions 1 and 2 which harbor all the predicted IgE epitopes in all food and pollen TLPs in either of the region. Further, based on the number of IgE epitopes, food TLPs are grouped into rosid and non-rosid clades. The number and distribution of the predicted IgE epitopes among the allergenic TLPs may explain the specificity of food or pollen allergy as well as the varied degree of cross-reactivity among plant foods and/or pollens.

The allergenic potential of sapodilla allergens in a mouse model was also investigated. BALB/c and Swiss-albino mice strains were injected intraperitoneally with various sapodilla proteins to observe the effects of challenge administrations following the initial sensitization; at the termination of the experiments, the collected serum was analyzed for IgG and IgE. Although ELISA for IgG and IgE in the sera of mice did not show any significant results, anaphylactic death was observed in the groups receiving the adjuvant alum, which indicates production of IgE. The simulated gastric fluid stability and thermal stability of sapodilla acidic TLP was assessed, which showed that the allergenic protein is stable upon heat treatment at 90°C up to 60 min, and retains its immunoreactivity with the allergic subjects’ sera pooled even after digestion up to 60 min. Sapodilla acidic TLP did not show any antifungal activity against selected fungal species. The polyclonal antisera raised against the prototype protein, thaumatin was found to recognize TLPs from sapodilla fruit. All these data described in Chapter 5 suggests that sapodilla acidic TLP has characteristics of an allergen.

Many future perspectives can be addressed in the field of fruit allergy, based on the important findings from this thesis. These are presented in Chapter 6, with an emphasis on sapodilla fruit and TLP allergens. The thesis concludes with bibliography (alphabetical), and a copy of the publications arising out of this investigation.