

ORAL PRESENTATION

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Biochemical and immunological characterisation of Act d 10 a lipid transfer proteins from green kiwifruit

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Aims

The aim of this study was to assess the presence of a Lipid Transfer Proteins (LTP) in green kiwifruit (Actinidia deliciosa) and to evaluated the allergenic activity of the newly identified protein.

Methods

Pulp and seeds of green kiwifruit were manually separated and homogenized. Act d 10 was purified from seed extracts by ion exchange chromatography and RP-HPLC, and sequenced by an automatic amino acid sequencer. Act d 10 was evaluated in vivo by skin prick test (SPT) and in vitro by detecting IgE using streptavidin-conjugated CAP (Phadia, Sweden). Act d 10 was spotted on a customized ISAC microarray (PMD, Austria) for evaluating IgE prevalence in a larger cohort of allergic patients.

Results

The purification procedure allowed to obtain about 0.4 mg of natural Act d 10 from 1 g of kiwifruit seeds. The primary structure comprises 92 amino acids with a molecular mass of 9,458 Da. Homology by BLAST search in Uniprot database showed Act d 10 displaying the higher identity (56%) with an isoform of Ara h 9. Act d 10 shares 47% of overall sequence identity with Pru p 3. However, higher levels of residue conservation are detected in some regions. Seven patients selected with clinical history of kiwifruit allergy and specific IgE to Pru p 3 were positive to Act d 10 by SPT; the same

patients had specific IgE to Act d 10 as detected by the CAP system. 804 randomly selected allergic patients were tested for IgE to Act d 10 and 170 of them (21.14%) were detected positive; 152 of these 170 patients (89.41%) had IgE to Pru p 3.

Conclusions

Act d 10 is a new LTP isolated from Actinidia deliciosa, contained in significant amounts in the green kiwi seed. The sequence identity between Act d 10 and other already known allergenic LTPs is not very high, however the significant conservation of some epitope regions suggested potential IgE cross-reactivities. The IgE binding activity of sera from clinically allergic patients has been shown by SPT and direct IgE detection on two different systems. IgE co-recognition of kiwi LTPs and other known LTPs seems to be dependent on the epitope distribution on the different allergens.

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