

## **IgE Reactivities of Ara h 1 Vicilin Buried Peptide and Purified Natural Ara h 1: Revisiting the Conformational Epitopes**

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**Rationale:** Vicilin buried peptides (VBPs) are present at the N-terminus of Vicilin storage proteins before being processed off. VBPs bind IgE antibodies and are believed to mediate allergic responses/cross-reactivity, through a conserved structural-fold. Delineating the molecular basis of cross-reactivity would provide valuable insight into the allergenic properties of VBPs.

**Methods:** nArah1 was purified from defatted peanut extract and rArah1-VBP and rCora11-VBP were expressed in *E. coli*. Proteins were purified using multi-step chromatography. Purity was assessed by Mass-spectrometry (LC-MS/MS), and the IgE-reactivities of recombinant-Arah1-VBP (rArah1-VBP) and purified natural-Arah1 (nArah1) were compared in chimeric ELISA-assay using 14 peanut-allergic sera. Cross-reactivity between peanut (rArah1-VBP) and hazelnut VBPs (rCora11-VBP) was compared using a polyclonal antibody against rArah1-VBP.

**Results:** Validation of the protein purities (>99%), and the absence of natural Arah1-VBP in the purified nArah1 preparations was confirmed by LC-MS/MS. IgE antibodies in all 14 sera reacted with nArah1 and 13/14 reacted to rArah1-VBP. Upon reduction/alkylation, both allergens retained IgE-reactivity, with 2/14 sera showing decreased IgE-reactivity to nArah1, and 7/13 sera decreased reactivity to rArah1-VBP. rCora11-VBP exhibited significant reactivity to the polyclonal antibody raised against rArah1-VBP.

**Conclusions:** Results indicate that Arah1-VBP has substantial IgE-binding reactivity independent of nArah1-vicilin, suggesting that rArah1-VBP can be useful for improved molecular allergy diagnostics. The pronounced loss in IgE-reactivity of the reduced rArah1-VBP points to the presence of a larger number of conformational epitopes on VBP relative to nArah1. IgG cross-reactivity between peanut and hazelnut VBPs, despite lack of sequence similarity, supports the importance of conformational epitopes in VBPs.