

The Effects of Glycosidic Treatment on the Levels and Allergenic Properties of the Major Peanut Allergens Ara h 1 and Ara h 2

H. Singh¹, S.J. Maleki² and M. J. Cantoria¹

¹School of Kinesiology and Nutritional Science, California State University, Los Angeles, CA 90032

²US Department of Agriculture, Food Allergy Unit, New Orleans, LA 70124

ABSTRACT

Allergen proteins in cereals and nuts function as storage proteins and possess similar general characteristics. The objective of this study was to alter the levels and reduce the allergenic properties of two major peanut allergens, Ara h 1 and Ara h 2, by cleaving their glyco-portion through enzymatic treatment. The Crude Peanut Extract (CPE) of raw, unshelled and unsalted US Virginia peanuts was incubated at 37°C with the glycosidase for 0h, 1h, 2h and 3h and studied for changes in their allergenic properties. RP-HPLC indicated a change in areas of certain peaks by as much as 100%. SDS-PAGE confirmed that the monomeric form of Ara h 1 (63.5kDa) and the Ara h 2 (17kDa) doublet decreased with enzymatic incubation. Glycosidase-treated CPE also showed less binding of Ara h 1 and Ara h 2 to chicken IgY and to human IgE. Glycosidic treatment appears to affect the integrity and allergenic properties of Ara h 1 and Ara h 2.

INTRODUCTION

- ▶ Ara h 1 and Ara h 2 have been considered major peanut allergens as they are recognized by IgE from greater than 90% of peanut-allergic patients.¹
- ▶ Ara h 1 and Ara h 2 are highly stable glycoproteins that are resistant to heat and proteases.²
- ▶ Ara h 1 is able to form a trimer that protects IgE-binding sites (called epitopes) from denaturants (Fig 1).³
- ▶ Ara h 2 belongs to the prolamin superfamily, together with the allergens in rice, wheat, barley and rye.²
- ▶ Ara h 2 (Fig 2) does not possess aggregatory behavior but is stabilized by disulfide bonds.⁴



Fig 1A. 3D structure of the major peanut allergen Ara h 1.

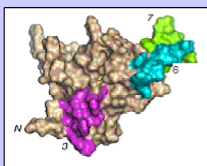


Fig 1B. 3D structure of the major peanut allergen Ara h 2.

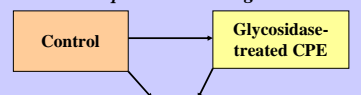
- ▶ The present study was an attempt to modify the allergenic properties of peanut allergens by using enzymatic treatment.

MATERIALS AND METHODS

CPE (Crude Peanut Extract) Preparation⁵

- ▶ Defatted ground peanuts with acetone and diethyl ether.
- ▶ Air-dried defatted peanuts for 24h.
- ▶ Stirred pellets in 0.1M NH₄HCO₃ (pH 8.0), 20h at 25°C.
- ▶ Centrifuged for 80min at 20, 200g at 4°C (2x).
- ▶ Filtered supernatant, freeze-dried and labeled as CPE.

Experimental Design



1. RP-HPLC⁶ - used for preliminary screening of general changes in the proteins of enzyme-treated CPE.

- ▶ C₁₈ 250 x 4.60mm 4 micron Jupiter 4μ Proteo; Phase A = 0.05% trifluoroacetic acid (TFA) in H₂O, Phase B = 0-100% gradient of 0.05% TFA in methanol, 1mL/min injection rate, 100μL injection volume, 280nm, 60min analysis

2. SDS-PAGE - used to separate, identify, detect the levels and degradation of peanut allergens.

- ▶ Sample dissolved in 3x loading buffer
- ▶ incubated for 10min at 65°C
- ▶ Separated in 4-20% Tris-HCl gel

3. IMMUNOBLOTTING - for the detection of changes in the levels of allergens and allergenicity.

- ▶ SDS-PAGE samples transferred to PVDF membrane
- The membrane was:
 - ▶ Blocked with Biotto
 - ▶ Incubated with diluted primary antibody for 1hr with chicken IgY or over night with human IgE
 - ▶ Washed with PBST 3x
 - ▶ Incubated with secondary antibody for 1h (anti-chicken IgY or anti-human IgE)
 - ▶ Washed with PBST 3x and PBS 2x
 - ▶ Incubated with ECL-Plus Western Substrate
 - ▶ Visualized using a CCD camera system

RESULTS AND DISCUSSION

RP-HPLC of Control vs Treated CPE

- ▶ Several changes in the areas of the peaks corresponding to the allergens were observed and became the basis in selecting samples for further analysis.

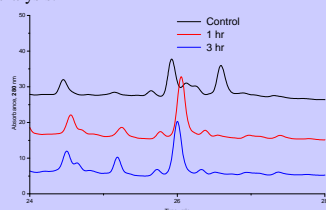


Fig 2. RP-HPLC profile of control and enzyme-treated CPE.

SDS-PAGE of Control vs Treated CPE

- ▶ Ara h 1 is degraded by the glycosidase immediately after enzymatic incubation (A, undigested control to E, 3h incubation with enzyme).
- ▶ Ara h 2 is degraded only after 1h incubation (C, 1h incubation with enzyme).
- ▶ Ara h 2 forms lower molecular weight migratory bands indicative of enzymatic digestion.

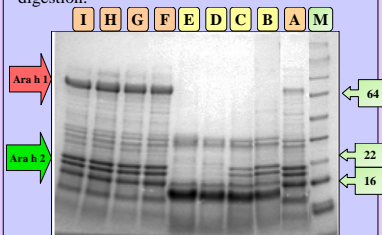


Fig 3. SDS-PAGE of peanut samples (MW in kDa) of control vs. glycosidase-treated CPE. Enzyme-treated CPE, incubation hours: A = Control, B = 0h, C = 1h, D = 2h, E = 3h. Untreated CPE Control, incubation hours: F = 0h, G = 1h, H = 2h, I = 3h

Immunoblot Test of Immunoreactivity

- ▶ Immunoblots (Fig 4) show that Ara h 1 is degraded by the enzyme, as supported by the SDS-PAGE result (Fig 3).

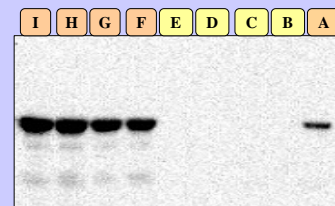


Fig 4. IgY-binding of Ara h 1 after enzymatic treatment. See Fig 3 for lane labels (A to I).

- ▶ Ara h 2 was degraded at a slower rate than Ara h 1 by the glycosidase (Fig 5). Its degradation started at 1h and significant breakdown followed at 2h and 3h.

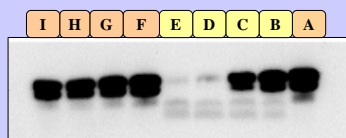


Fig 5. IgY-binding to Ara h 2 after enzymatic treatment. See Fig 3 for lane labels (A to I).

Immunoblot Test of Allergenicity

- ▶ A decrease in the IgE-binding is seen with both Ara h 1 and Ara h 2 as they are degraded with glycosidase.
- ▶ The degradation and therefore decrease in IgE binding was more obvious for Ara h 2.
- ▶ Enzyme-treated Ara h 1 was degraded and also exhibited a reduction in IgE-binding compared to the control.

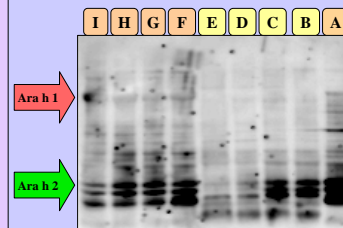


Fig 6. IgE-binding of Ara h 1 and Ara h 2 after enzymatic treatment. See Fig 3 for lane labels (A-I).

CONCLUSION

- ▶ This preliminary investigation suggests that glycosidic treatment was affecting the levels and potentially the allergenic properties of the major peanut allergens Ara h 1 and Ara h 2.
- ▶ It is possible that removal of the glycosylation residues of the peanut proteins makes them more susceptible to degradative enzymes within the peanut.

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