

Tertiary Structure and Biophysical Properties of a Major Peanut Allergen, Implications for the Production of a Hypoallergenic Protein

Gary A. Bannon David Shin Soheila Maleki Randall Kopper
A. Wesley Burks

Departments of Biochemistry and Molecular Biology and Pediatrics, University of Arkansas for Medical Sciences
and Arkansas Children's Hospital Research Institute, Little Rock, Ark., USA

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Peanut allergy is a significant health problem because of the prevalence and potential severity of the reaction. Ara h 1, a major peanut allergen recognized by serum IgE from >90% of peanut-sensitive patients, has been isolated and characterized [1, 2]. This allergen is 626 amino acids long and contains 23 linear IgE-binding epitopes, 6–10 residues in length [2, 3]. The amino acids important for peanut-specific IgE binding were determined by synthesizing wild-type and mutant peptides with single alanine, glycine or methionine substitutions at each position followed by incubation in pooled serum from patients with peanut hypersensitivity. From this analysis it was determined that amino acids which reside in the middle of the epitope were generally more critical for IgE binding. Furthermore, though polar charged residues occur most frequently within the epitopes, hydrophobic residues were found to be more important for IgE binding. In addition, it was found that each epitope could be mutated resulting in loss of ability to bind IgE with only a single amino acid substitution [4]. To further characterize the epitopes a homology-based molecular model of the Ara h1 protein was made. The model represents residues 171–586 allowing visualization of epitopes 10–23. The majority of these epitopes appear to be clustered to certain areas of the molecule. Many of the critical amino acids involved in binding are evenly distributed on the surface and not buried in the hydrophobic core [4].

Utilizing a fluorescence anisotropy assay and purified native Ara h 1 protein it was determined that the allergen forms a very stable higher order structure consisting of three Ara h 1 monomers that interact with each other via hydrophobic amino acid residues. A molecular model of the tertiary structure of the Ara h 1 monomer [4] indicated that the majority of the surface-accessible hydrophobic residues reside on the alpha helical ends of the molecule. The x-ray crystal structure of the highly homologous phaseolin trimer [5] was used to model the tertiary structure of the Ara h 1 trimer. The hydrophobic regions located on the arms at each end of the Ara h 1 protein form the contact points for monomer interactions. In addition, most of the IgE-binding epitopes are located at the contact points between the Ara h 1 monomers. These results reveal that both hydrophobic regions and the IgE-binding epitopes are clustered around the contact points for trimer formation. Amino acid substitutions designed to reduce the allergenicity of this protein could unintentionally disrupt trimer formation and protein function. These observations have important implication for studies designed to develop hypoallergenic proteins to replace native allergens.

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