

IgE-Binding Epitopes of Shrimp Tropomyosin, the Major Allergen Pen a 1

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Key Words

Recombinant allergen · Epitopes · Allergen structure · *Penaeus aztecus* · Crustacea · Major Allergen · Pen a 1

Introduction

The molecular basis of allergenicity remains unclear even though amino acid sequences and epitopes of numerous allergens have been determined in recent years. Tropomyosins belong to a family of highly homologous proteins that contains both allergens and nonallergens; tropomyosin from brown shrimp, *Penaeus aztecus*, has been identified as the major allergen, Pen a 1 [1], yet beef, pork and chicken tropomyosins are not allergenic [2]. Thus these molecules can aid our understanding of the relationship of protein structure to allergenicity.

Materials and Methods

In order to characterize Pen a 1 epitopes, a recombinant peptide library (Novatope epitope mapping system) was constructed and overlapping peptides (SPOTs system, Genosys) were synthesized. The Pen-a-1-coding plasmid was randomly cleaved by DNase I in the presence of Mn²⁺, causing double-strand cleavage. Electrophoretically separated fragments, averaging 50–150 bp in size, were eluted (QIAEX II Agarose Gel Extraction Kit, Qiagen), treated successively with T4 DNA polymerase and Tth DNA polymerase, ligated into the pTOPET vector and transfected into NovaBlue (DE3) cells. The library was screened with a serum pool of shrimp-allergic subjects and positive clones were sequenced. Forty-six overlapping peptides

(length: 15 amino acids, offset: 6 amino acids), spanning the entire Pen a 1 molecule, were synthesized on cellulose membranes, blocked and incubated with 1:5 diluted serum pool and one individual serum of shrimp-allergic subjects overnight. IgE reactivities were detected using ¹²⁵I-labeled horse anti-IgE (0.08 µCi/ml; Sanofi Diagnostics Pasteur, Inc.) and autoradiography.

Results

Four recombinant peptides and 9 synthetic peptides bound Pen-a-1-specific IgE, respectively (table 1), located at the N terminus, center and C terminus of the Pen a 1 molecule. Analysis of IgE binding of sequences that are identified by two different techniques and/or overlapping peptides, identified three major allergenic regions: Pen a 1 (119–148), Pen a 1 (153–179) and Pen a 1 (241–282), suggesting that the center and the C terminus of the shrimp tropomyosin molecule contain most of the IgE-binding sites. These results will provide a better understanding of the relationship of protein structure and allergenicity.

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Table 1. IgE-binding sites of Pen a 1, identified by recombinant and synthetic, overlapping peptides

Location	Recombinant peptide library		Overlapping, synthetic peptides	
N terminus			Pen a 1 1-15 Pen a 1 79-93	MDAIKKKMQAMKLEK SNAEGEVAALNRRIQ
Center	Pen a 1 157-169 Pen a 1 167-179 Pen a 1 136-148	EADRKYDEVARKL RKLAMVEADLER SDEERMDALENQL	Pen a 1 109-123 Pen a 1 121-135 Pen a 1 133-147 Pen a 1 187-201	ATTKLAEASQAAD DESERMRKVLENRSL RSLSDDEERMDALENQL ESKIVLEBEEELRWG
C terminus	Pen a 1 262-282	NEKEYKSITDELDTFSELS	Pen a 1 217-231 Pen a 1 241-255 Pen a 1 253-267	REEAYKEQIKTLTNK FAERSVQKLQKEVDR VDRLEDELVNEKEY

References

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