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Short Communication
1. Allergens, Epitopes and Allergen Recognition

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Characterization of Recombinant Shrimp Allergen Pen a 1 (Tropomyosin)

Gerald Reese
Byeoung-Ju Jeoung
Carolyn B. Daul
Samuel B. Lehrer

Clinical Immunology and Allergy Section, Department of Medicine, Tulane University Medical Center, New Orleans, La., USA

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Recombinant allergen
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Abstract
Tropomyosin (Pen a 1) from brown shrimp, *Penaeus aztecus*, has been identified as the only major shrimp allergen. Since beef, pork and chicken are other tropomyosin-containing foods that are not very allergenic, tropomyosins can serve to investigate the contribution of the structural properties of a protein to its allergenicity. The aim of this study was to determine the primary structure of Pen a 1 and to identify IgE-binding epitopes. The screening of a unidirectional expression cDNA library from shrimp tail muscle with the Pen-a-1-specific monoclonal antibody 4.9.5 resulted in 4 positive *Escherichia coli* clones. Immunoblot analysis with human sera from shrimp-allergic subjects demonstrated IgE binding of all 4 recombinant shrimp proteins. Three of 4 expressed recombinant proteins have a molecular weight of approximately 36 kD, consistent with the molecular weight of natural Pen a 1. The DNA sequence analysis identified these recombinant shrimp proteins as tropomyosin and could be aligned with the sequence of greasyback shrimp (*Metapenaeus ensis*) tropomyosin (Met e 1). In order to characterize contiguous IgE-binding epitopes of Pen a 1, a peptide library (Novagen epitope mapping system) expressing 10–30 amino-acid-residue-long recombinant Pen a 1 peptides was constructed and screened with human IgE. Four recombinant, IgE-reactive Pen a 1 peptides were selected and sequenced. They show various degrees of sequence identity with tropomyosins of other arthropods, such as fruitfly and house dust mite, helminths and vertebrates.

Introduction

The molecular basis of protein allergenicity still remains unclear. Most potent food allergens are heat and acid resistant, occur in substantial quantities and are usually glycosilated. However, these characteristics also describe many nonallergens. The sequences of numerous allergens have been determined in recent years, and IgE-binding sites and T-cell-reactive sequences have been identified, yet no general principle could be deduced to predict these sequences. Tropomyosins are a family of highly homologous proteins that contain both allergens and nonallergens. The primary structures of tropomyosins from a large variety of origins have been determined and tropomyosins of different shrimp species (*Penaeus aztecus, Penaeus indicus, Metapenaeus ensis*) have been identified as major shrimp allergens [1–4]. Cross-reacting Pen a 1-like allergens were detected in other crustacea species [5, 6]. Since beef, pork and chicken are other tropomyosin-containing foods that are not very allergenic, tropomyosins can serve to investigate the contribution of the structural properties of a protein to its allergenicity. Determining the primary structure of Pen a 1 and identifying IgE-binding epitopes are the first steps into this direction.

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Correspondence to: Dr. Gerald Reese
Tulane University Center, Department of Medicine
Clinical Immunology and Allergy Section
1700 Perdido Street, New Orleans, LA, 70112 (USA)
Tel. (504) 588 5578, Fax (504) 584 3686

Materials and Methods

cDNA Library Construction and Screening

PolyA mRNA was purified with the PolyATtract® system (Promega, Madison, Wisc., USA) from total RNA isolated [7] from the tail muscle of snap-frozen brown shrimp. The cDNA library was constructed as a unidirectional cDNA library using the phagemid vector (pcDNA II, Invitrogen, San Diego, Calif., USA) and was screened with the Pen-a-1-specific monoclonal antibody (mAb) 4.9.5. Positive clones were tested for IgE reactivity by SDS-PAGE and immunoblotting and their cDNA sequences determined.

Peptide Library Construction

The plasmid of clone P7, coding for Pen a 1, was purified (Plasmid Purification Kit, Qiagen, Chatsworth, Calif., USA) and the peptide library was constructed using the Novatope epitope mapping system (Novagen, Madison, Wisc, USA). Briefly, the plasmid was randomly cleaved by DNase I in the presence of Mn^{2+}, causing double-strand cleavage. The DNA fragments, were fractionated on an 2% agarose gel. Fragments, averaging 50–150 bp in size, were eluted (QIAEX II Agarose Gel Extraction Kit, Qiagen) and treated successively with T4 DNA polymerase and Tth DNA polymerase, which repaired and then added a single dA residue to each 3′ end. The DNA fragments were ligated into the pTOPE T vector containing single dT overhangs and transfected into NovaBlue(DE3) cells. The library was screened with a serum pool of shrimp-allergic subjects.

Results

Immunological Characterization of Recombinant Pen a 1 Peptides

All 4 recombinant proteins (P1, P6, P7, P10), selected with the Pen-a-1-specific mAb bound human IgE. The molecular weights of P6, P7 and P10 were virtually identical with the molecular weight of natural Pen a 1. The lower molecular weights of P1 and P6 correspond to their truncated cDNA sequences. The P1 protein has a molecular weight of approximately 10 kD (fig. 1).

Sequence Comparison of Pen a 1 and Met e 1

The cDNA and the deduced amino acid sequences identified all four recombinant proteins as the muscle protein tropomyosin. P7 and P10 have identical cDNA and amino acid sequences whereas P6 has a 93 bases/31 amino acid 5′/N-terminal deletion. When P6, P7 and P10 were compared with Met e 1, 26 base substitutions were detected. Only one of them resulted in an amino acid substitution. Ninety bases/30 amino acids of P1 could be aligned with the 3′/C-terminus of Met e 1. In this short stretch of cDNA, 26 base pair substitutions were detected that result in 7 amino acid substitutions (fig. 2).

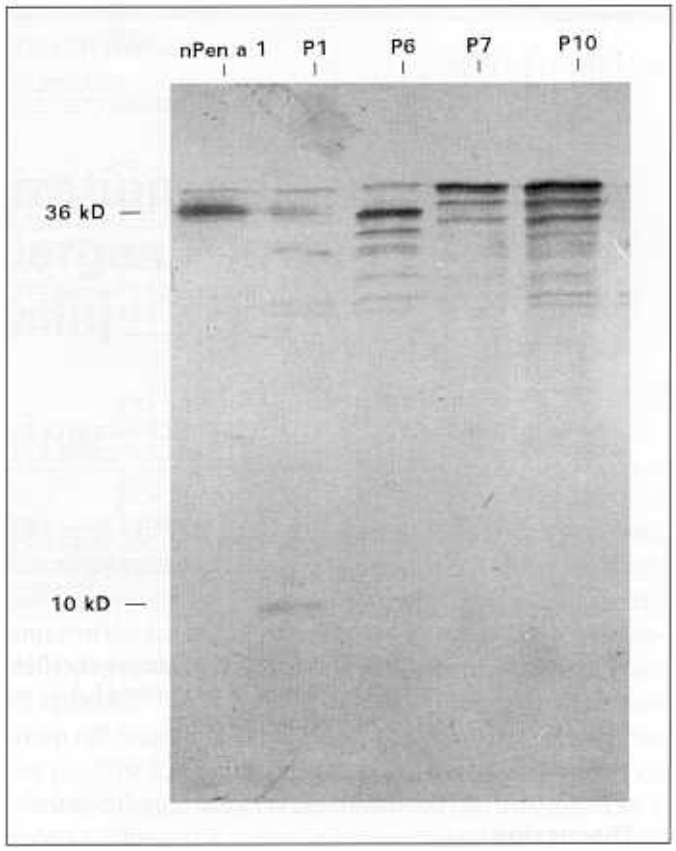

Fig. 1. Comparison of IgE reactivities to natural Pen a 1 (nPen a 1) and recombinant Pen a 1 clones P1, P6, P7 and P10.

IgE-Reactive, Recombinant Peptides

Four IgE-reactive peptides (E2, E3, E4, E6) were sequenced and aligned with the sequence of Pen a 1 and Met e 1. Three (E2, E3, E6) peptides are 13 and 1 (E4) is 21 amino acid residues long. The sequences of peptides E2 and E6 overlap by 3 amino acid residues. An IgE-reactive peptide, previously identified in Indian shrimp, *P. indicus* [8], partially overlaps with E6 (fig. 2). The sequence of E2 is 100% identical with those of tropomyosins of greasyback shrimp, *M. ensis*, other arthropods (*Drosophila melanogaster, Locusta migratoria, Dermatophagoides farinae*) and helminths (*Trichostrongylus colubriformis, Caenorhabditis elegans, Onchocerca volvulus*). Vertebrate tropomyosins show sequence identity between 53% (*Gallus gallus*) and 69% (*Rattus norvegicus*, *Homo sapiens*, *Sus scrofa*) with E2. The sequence of E6, however, is very similar (92%) with various vertebrate tropomyosins. On the other hand, only the homologous *M. ensis* tropomyosin is identical with the E4 sequence; the sequence identity with arthropods and helminths are lower than those with other Pen a 1 peptides and range from 57% (*C. elegans*) to 80% (*D. farinae*).

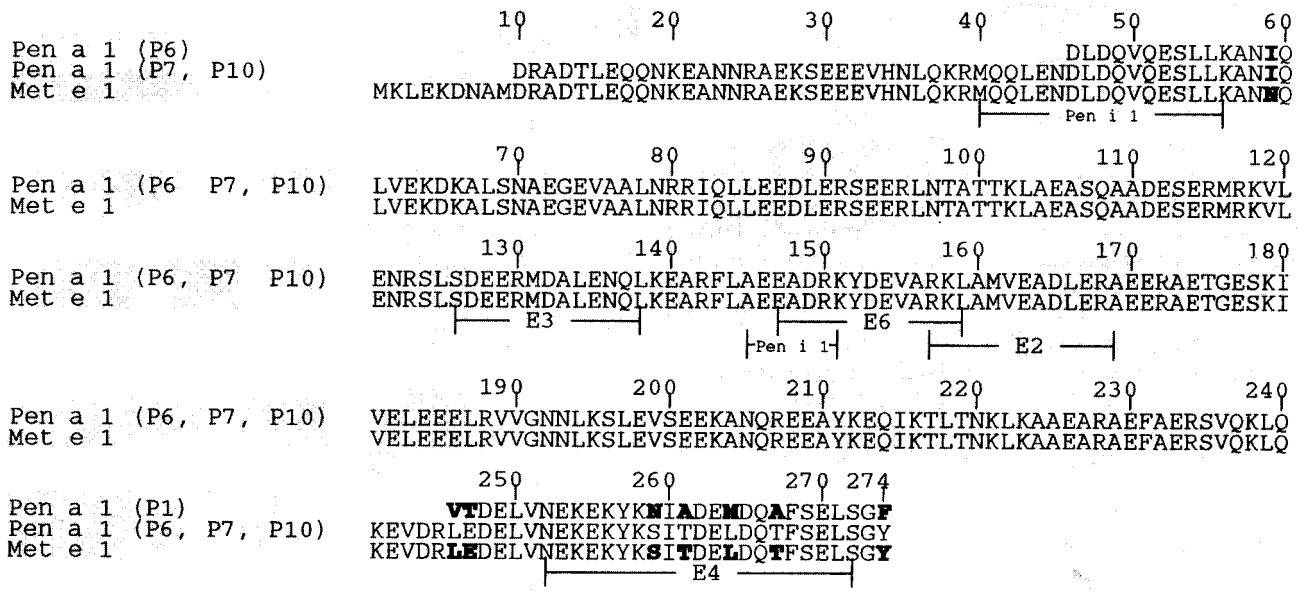

Fig. 2. Amino acid alignment of cDNA sequences of recombinant Pen a 1 (P1, P6, P7, P10) with tropomyosin of greasyback shrimp *M. ensis*, Met e 1. Amino acid substitutions are bolded. IgE-reactive Pen a 1 (E2, E3, E4, E6) and Pen i 1 [8] peptides are marked.

Discussion

Four IgE-reactive, recombinant peptides of the major allergen Pen a 1 from brown shrimp, *P. aztecus*, were sequenced. All peptides are located in the second half of the molecule including the C terminus. An IgE-reactive peptide, previously identified in Indian shrimp, *P. indicus* [8], partially overlaps with E6, indicating that this sequence of shrimp tropomyosins is a major IgE-binding site. The epitopes are located in both phylogenetically diverse and conserved parts of the tropomyosin molecule. This is in good agreement with the allergenic activity of other tropomyosins. However, no IgE-reactive Pen a 1 peptide shows complete sequence identity with vertebrate tropomyosins. Since tropomyosins are a family of highly homologous proteins that contain both allergens and nonallergens and the sequences of tropomyosins from a large variety of origins have been determined, tropomyosin is a good model to study the contribution of the primary structure to the allergenicity of proteins.

Acknowledgments

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