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The IgE-Binding Regions of the Major Allergen Pen a 1: Multiple Epitopes or Intramolecular Cross-Reactivity?

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Invertebrate allergens · Crustacea · Tropomyosin · IgE-binding peptides

Abstract
The muscle protein tropomyosin is the major allergen (Pen a 1) in brown shrimp, *Penaeus aztecus*, lobster, crab, mollusks and other invertebrates such as house dust mite, and cockroach. Pen a 1 contains five major IgE-binding regions, and sequences that are rarely or never recognized by IgE antibodies from shrimp-allergic subjects. The molecular structure of tropomyosins is simple and repetitive; it is made up of multiple heptad repeats. The analysis of sequence identities and similarities of major, minor and non-IgE-binding regions indicate that intramolecular cross-reactivity to the multiple IgE-binding regions of Pen a 1 is not due to repetitive, identical or similar, cross-reacting sequences but due to multiple, independent epitopes. However, an allergenic motif may explain the reactivities to the five identified major IgE-binding regions.

Introduction

Crustaceans are a frequent cause of allergic reactions to foods [1] such as shrimp, crab, crawfish and lobster. Several allergens were identified, however, the muscle protein tropomyosin seems to be the only major allergen. In brown shrimp, *Penaeus aztecus*, at least 80% shrimp-allergic subjects react to Pen a 1 and this protein binds at least 85% of the shrimp-specific IgE from shrimp-allergic subjects [2]. In addition, tropomyosins are important allergens in other crustaceans, mollusks and in other invertebrates such as house dust mites and cockroaches [3]. In a previous study [4], the major and minor IgE-binding regions of Pen a 1 were located by testing 18 sera from shrimp-allergic subjects for IgE antibody reactivity to 46 synthetic, overlapping peptides (length 15 amino acids, offset 6 amino acids) spanning the whole length of the tropomyosin molecule. Briefly, based on frequency and intensity of the individual IgE reactivities, Pen a 1 contains five major IgE-binding regions [region 1: Pen a 1 (43–57), region 2: Pen a 1 (85–105), region 3: Pen a 1 (133–153), region 4: Pen a 1 (187–207), region 5: Pen a 1 (247–284)]. In addition, 22 peptides were categorized as minor IgE-binding regions, and 12 peptides did not bind any IgE antibodies. Since the molecular structure of tropomyosins is quite simple and repetitive (the tropomyosin monomer contains a heptad repeat $(abcdefg)_n$ in which generally large hydrophobic nonpolar residues occur at positions a and d, while positions b, c, e, f and g are usually occupied by polar or ionic amino acids [5]), the question arises whether the presence of multiple IgE-binding regions is due to repetitive, identical or similar, cross-reacting sequences or due to multiple, independent epitopes. To approach this question, sequence identities of major, minor and non-IgE-binding regions were calculated and compared with each other.

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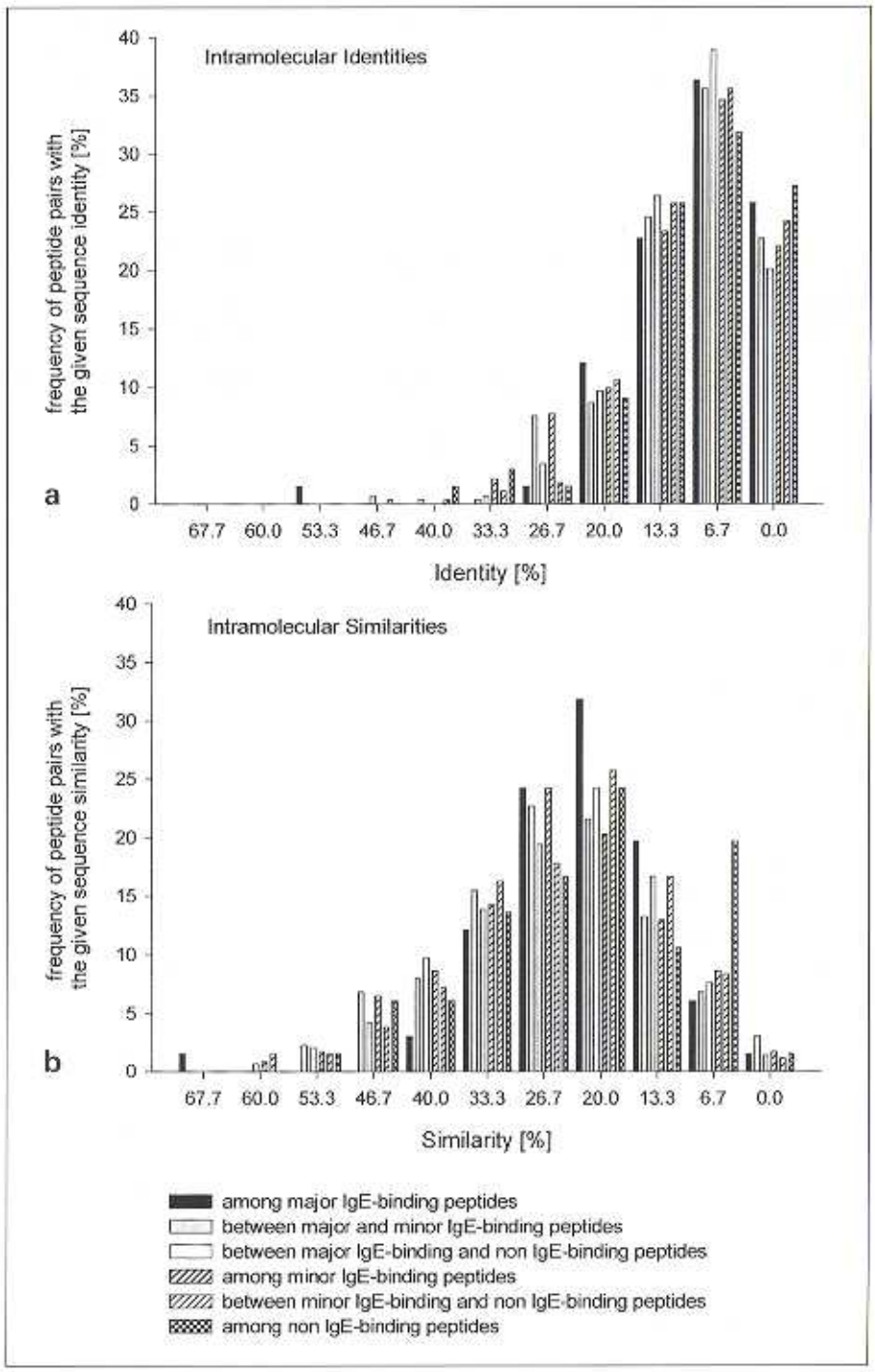

Fig. 1. Distributions of frequencies of intramolecular identities (**a**) and similarities (**b**).

Material and Methods

Sequence Identities and Similarities

Sequence identities (same amino acids in identical positions) and similarities (same or similar amino acids identical in identical positions) within each group of peptides (major, minor and non-IgE-binding regions) were calculated. For the determination of sequence similarities amino acids were grouped according to their properties into non-polar, aliphatic (glycine, alanine, valine, leucine, isoleucine, proline), polar, uncharged (serine, threonine, cysteine, methionine, asparagine, glutamine), aromatic (phenylalanine, tyrosine, tryptophan), positively charged (lysine, arginine, histidine), and negatively charged (aspartate, glutamate) amino acids.

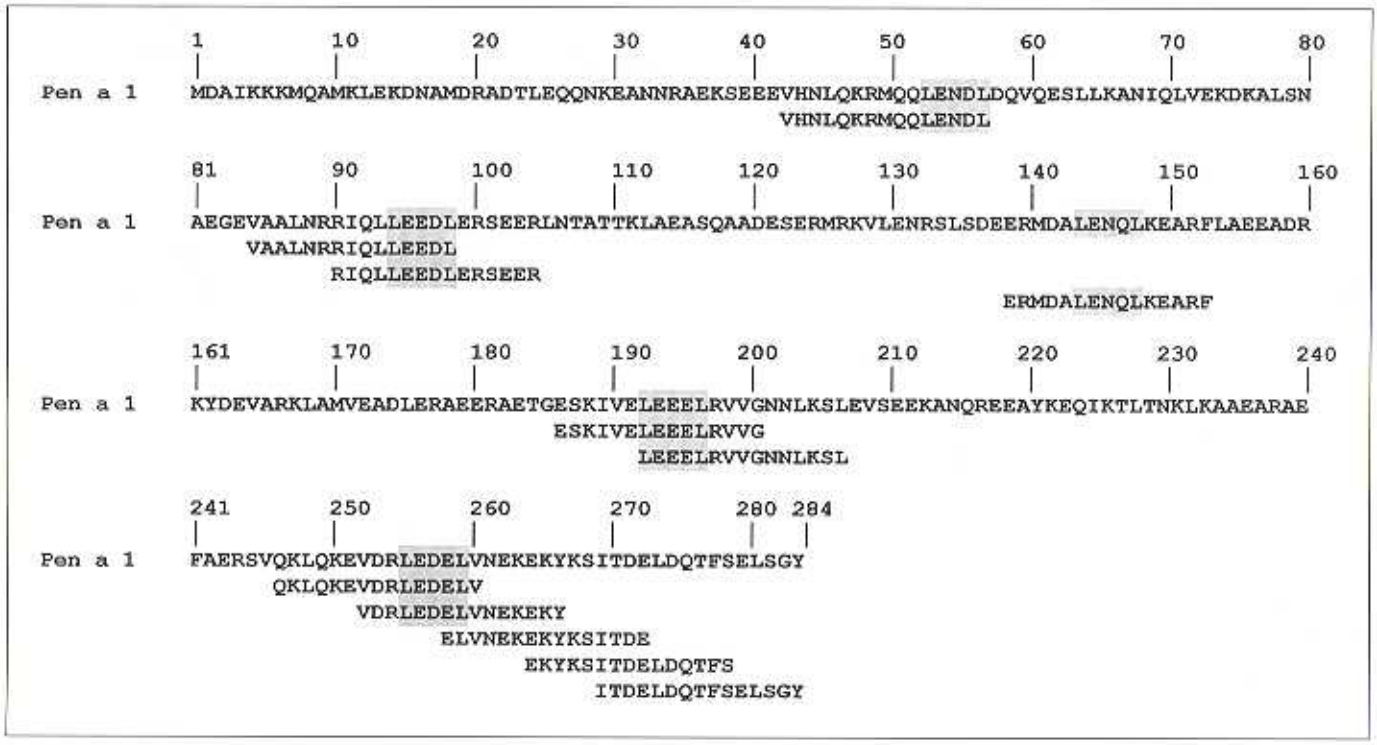

Fig. 2. The five major IgE-binding regions of Pen a 1 containing the LEXXL motif.

Statistical Analysis

Since the calculated sequence identities and similarities were not normally distributed the Kruskal-Wallis one-way analysis of variance on ranks was performed instead of one-way analysis of variance to test for statistical significant differences. If the differences in the median among the groups were greater than would be expected by chance, all pairs of groups were compared to isolate the group or groups that differ from the others using the multiple comparison procedure according to Dunn.

Results

Intramolecular Identities

Figure 1a shows the distributions of frequencies of the intramolecular identities; for example, for the comparison of major and minor IgE-binding peptides the sequence identities of all combinations between a major and a minor IgE-binding peptide was calculated. Since the peptides were 15 amino acids long, a difference of an amino acid in one position decreased the identities by 6.7% (1/15) resulting in possible identities of 0.0, 6.7, 13.3, 20.0, 26.7, 33.3, 40.0, 46.7, 53.3, 60.0, 67.7, 73.3, 80.0, 86.7, 93.3 and 100.0%, respectively. The statistical analysis showed that there is no statistical difference among the three groups of peptides; in regard to sequence identities, major IgE-binding peptides cannot be distinguished from minor or non-IgE-binding peptides.

Intramolecular Similarities

Figure 1b shows the distributions of frequencies of the intramolecular similarities. The statistical analysis showed a difference among the three groups of peptides. Using the multiple comparison procedure according to Dunn revealed a difference in frequency patterns between major and minor IgE-binding peptides; however, no difference was detected between major or minor IgE-binding peptides and non-IgE-binding peptides.

Allergenic Motifs

In previous studies, the presence of tandem amino acid repeats contained within allergens has been reported [6, 7]. All five major IgE-binding regions of Pen a 1 contain the amino acid sequence LEXXL, where L is leucine and X is usually a negatively charged amino acid such as glutamic acid (E) or aspartic acid (D). In regions 1 and 3, X may be an aspartic acid (D), glutamine (Q) or asparagine (N) (fig. 2). The tandem motifs are located at posi-

tions 53–57, 95–99, 144–148, 193–197 and 256–260. All LEXXL tandem repeats were found within the five major Pen a 1 IgE-binding regions.

Discussion

Based on the observations of a previous study [4] that identified major and minor IgE-binding, and non-IgE-binding regions, and the simple and repetitive structure of the major shrimp allergen Pen a 1 (tropomyosin) [5], the question arises whether the presence of multiple IgE-binding regions is due to repetitive, identical or similar, cross-reacting sequences within the Pen a 1 molecules or due to multiple, independent epitopes. To answer to this question, sequence identities and similarities of major, minor and non-IgE-binding regions were calculated and compared with each other. Furthermore, the major IgE-binding regions were analyzed for repetitive motifs. The analysis for possible intramolecular identities and similarities showed that the sequence identities and similarities among the major IgE-binding peptides are not larger than those among minor or non-IgE-binding regions, respectively, indicating that intramolecular homology cannot explain the multiple IgE-binding regions of Pen a 1. However, an allergenic motif, LEXXL, was found within the five major Pen a 1 IgE-binding regions. This motif was not found in any other part of the Pen a 1 molecule; it is an exclusive property of the parts of the Pen a 1 molecule that seem to carry the majority of the Pen a 1-specific IgE reactivity. In conclusion, it is necessary to identify the minimal IgE-binding sites (epitopes) within each IgE-binding region and compare their structural properties with those of nonallergenic sequences of Pen a 1 since sequence identities and similarities among the major IgE-binding peptides are not larger than those among minor or non-IgE-binding regions.

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