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Antigenic Analysis (IgE and Monoclonal Antibodies) of the Major Shrimp Allergen Pen a 1 (Tropomyosin) from *Penaeus aztecus*

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Abstract
Pen a 1, the major shrimp allergen from the brown shrimp *Penaeus aztecus* was purified by preparative SDS-PAGE. Peptides were generated from Pen a 1 by CNBr cleavage and endoproteinase (Lys-C, Glu-C, trypsin, alkaline protease, Arg-C, chymotrypsin) digestion. The molecular weights of the resulting CNBr cleavage and enzymatic digestion products, separated by peptide SDS-PAGE, ranged from 1.5 to 20 kD. Following SDS-PAGE and semidry blotting, the analysis of monoclonal antibody (mAb) and subjects' IgE reactivities demonstrated that with the exception of alkaline protease, all cleavage procedures yielded IgE-binding peptides. However, since not all peptides of every digest bind IgE, it appears that IgE-binding epitopes are restricted to certain parts of the Pen a 1 molecule. mAbs bound to CNBr, Lys-C, trypsin, Glu-C and Arg-C peptides. Since mAbs reacted to several peptides from the same digest, Pen a 1 may have several similar epitopes. The comparison of IgE and mAb reactivities demonstrated similar but not identical binding patterns.

Introduction

Immunoblot analysis of IgE reactivities had identified at least 13 IgE-binding bands in shrimp. Eighty-two percent of the sera bound to a 36-kD band. This protein, designated Pen a 1, is the only major shrimp allergen [1]. Pen a 1-like 36-kD protein bands were also detected in other Crustacea such as crawfish *(Procambarus clarkii)*, crab *(Callinectes sapidus)* and lobster *(Panulirus argus)* suggesting a common Crustacea allergen [2, 3]. The sequence of a 21-amino-acid residue peptide identified Pen a 1 as the muscle protein tropomyosin (60–87% sequence homology) [4, 5]. The aims of this study were to identify IgE-binding Pen a 1 peptides and analyze IgE and Pen a 1-specific monoclonal antibody (mAb) reactivities to Pen a 1 fragments by cleaving purified Pen a 1 with CNBr and endoproteinases and analyzing Pen a 1 fragments with IgE and Pen a 1-specific mAbs by immunoblot.

Material and Methods

Serum

Serum was collected from atopic shrimp-sensitive individuals, all with a history of respiratory, dermatologic, or gastrointestinal symptoms occurring within 1 h of shrimp ingestion. All individuals were skin test positive (wheal ≥3 mm) with shrimp extract and demonstrat-

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ed elevated levels of IgE antibodies (radioallergosorbent test binding ≥3%) to shrimp extract.

Production of mAbs

BALB/c mice were immunized twice, on days 1 and 7, with 5 µg of purified Pen a 1. After 5 weeks, spleen cells were fused with P3X63Ag8.U1 myeloma cells (ATCC CRL 1597) using the PEG fusion technique [6, 7]. Cells were screened for antibody-producing hybridomas by ELISA, checked for specificity by immunoblotting and cloned by limiting dilution.

Purification of Pen a 1

Pen a 1 was purified by preparative SDS-PAGE (Model 491 Prep-Cell, BioRad). 4 mg shrimp extract [8] were separated on the 28-mm ID column using Laemmli's discontinuous SDS-PAGE buffer system [9]. A 15-mm-high stacking gel (5%T, 1.5%C) was poured on top of the 60-mm-high separation gel (10%T, 1.5%C). The SDS-PAGE was run at 12 W constant power. 2.5-ml fractions were collected at a flow rate of 1.0 ml/min after the dye front eluted from the gel. The fractions were analyzed by SDS-PAGE and immunoblotting using a serum pool of shrimp-allergic subjects and Pen a 1-specific mAbs. Fractions that contained pure Pen a 1 were pooled, dialyzed and lyophilized.

Peptide Production

100 µg Pen a 1 were incubated overnight at room temperature in 10% CNBr in 70% formic acid in the dark [10]. CNBr products were lyophilized. 100 µg Pen a 1 were denatured in 50 µl denaturation buffer (50 m*M* Tris/HCl pH 8.0, 6 *M* urea, 5 m*M* DTT) followed by 1:8 dilution in protease buffer (Lys-C: 25 m*M* Tris/HCl pH 7.7, 1 m*M* EDTA; Glu-C I: 50 m*M* NH_4CH_3COO/CH_3COOH pH 4.0; Glu-C II: 50 m*M* Na_2HPO_4/NaH_2PO_4 pH 7.8; trypsin: 50 m*M* Tris/HCl pH 7.6, 1 m*M* $CaCl_2$; alkaline protease: 50 m*M* Tris/HCl pH 9.0, 5 m*M* $CaCl_2$; Arg-C: 50 m*M* Tris/HCl pH 7.5, 1 m*M* $CaCl_2$, 2 m*M* DTT; chymotrypsin: 50 m*M* Na_2HPO_4/NaH_2PO_4 pH 7.0). 1 µg of enzyme was added and Pen a 1 was digested overnight at 37°C.

Peptide SDS-PAGE and Immunoblotting

The buffer system of Schägger and von Jagow [11] was used with slight modifications. A 10-mm-high stacking gel (5%T, 2.5%C) was poured on top of the 60-mm-high separation gel (17.5%T, 2.5%C). The cleavage products were diluted in sample buffer and boiled for 5 minutes. After electophoresis, the peptides were transferred [12] onto CNBr-activated nitrocellulose membranes [13]. The individual IgE and mAb reactivities were detected using alkaline-phosphatase-labeled, monoclonal antihuman IgE (Southern Biotechnology Associates) and goat antimouse IgG+IgM (Jackson Immunoresearch Laboratories) in combination with the chemiluminescence substrate CSPD (Tropix).

Results

The preparative SDS-PAGE yielded pure and IgE-reactive Pen a 1. All cleavage procedures, with the exception of alkaline protease digestion, resulted in IgE-binding peptides. Not all Pen a 1 fragments bound IgE or mAbs. 7/8 shrimp-allergic subjects reacted with Pen a 1 peptides. The IgE-binding intensity and frequency varied by subject and peptide digest. For example, Arg-C fragments bound IgE of 6 subjects whereas trypsin fragments were detected by only 3 subjects; individual subjects reacted with up to 7/8 Pen a 1 digests. Depending on the digest, the IgE-binding peptides had a molecular weight as high as 20 and as low as 4 kD. 6/9 of the Pen a 1-specific mAbs reacted with Pen a 1 peptides and all of them bound to IgE-binding peptides. 6/8 cleavage procedures (CNBr, Lys-C, Glu-C I, Glu-C II, trypsin, Arg-C) produced both IgE- and mAb-binding peptides whereas the digestion with chymotrypsin produced only IgE-binding peptides. IgE and mAb reactivities showed similar but not identical binding patterns (fig. 1). An 8-kD CNBr peptide was detected by both mAb 8 and IgE whereas a 14-kD peptide was only detected by mAb 8. The mAb 1–4 showed their strongest reactivities to an 18-kD Lys-C fragment, whereas the most intense IgE binding occurred to 10- to 18-kD fragments.

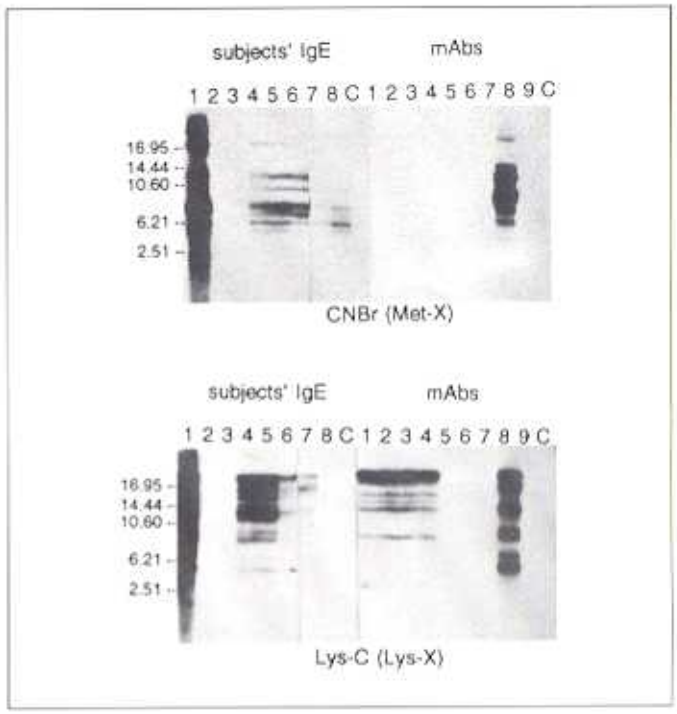

Fig. 1. Comparison of IgE and mAb reactivities to CNBr- and Lys-C-cleavage-derived Pen a 1 fragments.

Discussion

Immunologically active, IgE-binding Pen a 1, purified by preparative SDS-PAGE, was cleaved by CNBr and endoproteinases Lys-C, Glu-C, trypsin, alkaline protease, Arg-C and chymotrypsin. With the exception of alkaline protease digestion, all cleavage procedures resulted in IgE-binding peptides but not all peptides generated bound IgE. This indicates that IgE-binding epitopes are restricted to certain parts of Pen a 1. CNBr, Lys-C, trypsin, Glu-C and Arg-C cleavage produced mAb-binding peptides. In contrast to IgE reactivities, chymotrypsin fragments were not detected by any mAb. Since the same mAb reacted to different peptides from the same digest, Pen a 1 may have several similar epitopes. The comparison of IgE and mAb reactivities demonstrated similar but not identical binding, indicating that these Pen a 1-specific mAbs and subjects' IgE bound to different epitopes. In conclusion, Pen a 1 peptides generated by CNBr cleavage and enzymatic digestion contain IgE- and mAb-reactive epitopes. Further sequence analysis and epitope mapping of Pen a 1 and other tropomyosins will advance our understanding of the molecular basis and pathogenesis of shrimp allergy in particular and food allergy in general.

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