

Original Article

Identification of tropomyosin as a major allergen in the octopus *Octopus vulgaris* and elucidation of its IgE-binding epitopes

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ABSTRACT: The major allergen (named Oct v 1) in the muscle of the octopus *Octopus vulgaris* was purified by gel filtration on Sephacryl S-300, anion-exchange fast protein liquid chromatography on Mono Q and reverse-phase high-pressure liquid chromatography on TSKgel Octadecyl-4PW. In addition to the molecular mass, amino acid composition and cross-reactivity with Tur c 1 (turban shell *Turbo cornutus* allergen), the determined partial amino acid sequence clearly demonstrated that Oct v 1 is tropomyosin, similar to the known molluscan and crustacean allergens. Using peptide fragments isolated from the lysylendopeptidase digest of Oct v 1, competitive enzyme-linked immunosorbent assay inhibition experiments showed that IgE-binding epitopes of Oct v 1 are contained in two peptides (77–112 and 148–160) in the central region and one peptide (269–281) in the C-terminal region. In the peptide 77–112, the same sequence as the IgE-binding epitope proposed for Cra g 1 (oyster *Crassostrea gigas* allergen) is recognized at 92–105. Moreover, the peptide 148–160 partly overlaps with the IgE-binding epitopes suggested for Pen i 1 (shrimp *Penaeus indicus* allergen) and Pen a 1 (shrimp *Penaeus aztecus* allergen), and the peptide 269–281 with those for Tur c 1 and Pen a 1.

KEY WORDS: IgE-binding epitope, *Octopus vulgaris*, octopus, tropomyosin.

INTRODUCTION

Seafood allergy is reported frequently in coastal countries including Japan. Indeed, seafood ranks first among various causes of food allergy in Japanese adult patients, according to the survey performed in 1997 by the Ministry of Health and Welfare of Japan. Extensive studies with the codfish *Gadus callarias*^{1,2} and subsequent studies with several different species of fish^{3–5} have shown that the major fish allergen is parvalbumin. Alternatively, the major crustacean allergen is tropomyosin, as demonstrated with three species of shrimp (*Penaeus indicus*,^{6,7} *P. aztecus*⁸ and *Metapenaeus ensis*⁹), two species of lobster (*Pan-*

*ulinus stimpsoni*¹⁰ and *Homarus americanus*¹⁰), and one species of crab (*Charybdis feriatus*¹¹). Interestingly, immunoblotting studies revealed the cross-reactivity of patient sera between crustacean and molluscan allergens.¹² In accordance with this, we have isolated two isomeric allergens from the oyster *Crassostrea gigas*^{13,14} and one allergen from the turban shell *Turbo cornutus*,¹⁵ and have identified all of them as tropomyosins. Independently, the major allergen in the squid *Todarodes pacificus* has also been demonstrated to be tropomyosin by Miyazawa *et al.*¹⁶

It is essential to elucidate IgE-binding epitopes of food allergens, not only for the biochemical understanding of their allergenicity but also for the future development of hypoallergenic food products. In relation to this, it is worth noting that allergens in both crustaceans and mollusks are commonly tropomyosins but that their IgE-binding epitopes appear not to be necessarily common. Even for the two shrimp allergens, Pen i

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1 from *P. indicus*⁷ and Pen a 1 from *P. aztecus*,¹⁷ different segments were proposed as the IgE-binding epitopes. In addition, the IgE-binding epitopes reported for Cra g 1 (oyster allergen)¹⁴ and Tur c 1 (turban shell allergen)¹⁵ differed from each other and also differed from those for both Pen i 1 and Pen a 1. At present, no information about the IgE-binding epitopes of cephalopod allergens is available. In the present study, therefore, we selected the octopus *Octopus vulgaris*, which is consumed in Japan as commonly as squid but has not been examined for allergens. Attempts were made to purify and identify the major allergen of *O. vulgaris* and to elucidate its IgE-binding epitopes.

MATERIALS AND METHODS

Sera

Sensitized human sera were obtained from three subjects (subjects 1–3) who had a documented clinical history of immediate hypersensitivity reactions, such as asthma and diarrhea, after ingestion of crustaceans and mollusks. Using the radioallergen sorbent test, all subjects were diagnosed as having elevated serum IgE to these seafoods. Control serum was obtained from one subject (subject 4) without any adverse reactions after ingestion of seafood. These sera were stored at –20°C until required for use.

Enzyme-linked immunosorbent assay

Immunoglobulin E reactivity of allergens was examined using enzyme-linked immunosorbent assay (ELISA) as described elsewhere.¹³ Briefly, the allergens immobilized on a microplate (ELISA Plate H type; Sumitomo Bakelite, Tokyo, Japan) were immunoreacted with allergen-specific IgE antibodies in the sensitized sera (diluted 1 : 50), followed by peroxidase-conjugated goat antihuman IgE antibody (diluted 1 : 2500; Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA). Enzyme reaction was performed in 0.05 M phosphate-citrate buffer (pH 5.0) containing 0.1% *o*-phenylenediamine (Sigma Chemical Co., St Louis, MO, USA) and 0.03% H₂O₂. The developed color was measured by absorbance at 490 nm using a microplate reader (Model 450; Bio-Rad Laboratories, Hercules, CA, USA).

Cross-reactivity of the sera between the purified octopus allergen and Tur c 1 and IgE-reactivity of enzymatic fragments from the purified octopus allergen were assessed using the competitive ELISA inhibition method. The sensitized sera (diluted

1 : 25) were incubated with an equal volume of inhibitor solution (1 µg/mL) at 37°C for 1 h. A 0.1-mL portion of the mixture was then added to a microplate that had been coated previously with the purified octopus allergen (100 ng/well). The procedure followed thereafter was the same as for ELISA.

All ELISA experiments were performed in triplicate and the data were given in mean values.

Purification procedure

Throughout the purification procedure described below, proteins were traced by absorbance at 280 nm and allergens by ELISA using the serum from subject 1 as a sensitized serum and the serum from subject 4 as a control. The fresh muscle (40 g) from the most commonly edible part (brachium) of the octopus was extracted with 160 mL of 0.15 M NaCl–0.01 M phosphate buffer (pH 7.0). The crude extract was heated in a boiling water bath for 10 min. After removal of any insoluble matters formed, the supernatant was dialyzed against distilled water and lyophilized. The dried product was dissolved in 10 mL of 0.15 M NaCl–0.01 M phosphate buffer (pH 7.0) and applied to gel filtration on a Sephacryl S-300 column (2.5 cm × 95 cm; Pharmacia, Uppsala, Sweden) with the same solvent. Fractions of 10 mL were collected at a flow rate of approximately 30 mL/h. Allergen-containing fractions were combined, dialyzed against distilled water and lyophilized. The lyophilisate was dissolved in 10 mL of 0.01 M Tris-HCl buffer (pH 8.0) and subjected to anion-exchange fast protein liquid chromatography (FPLC) on a Mono Q HR 5/5 column (0.5 cm × 5 cm; Pharmacia). Elution was performed with a linear gradient of NaCl (0.0–1.0 M in 50 min) in the same Tris-HCl buffer at a flow rate of 1 mL/min. Allergenic fractions were combined and finally applied to reverse-phase high-pressure liquid chromatography (HPLC) on a TSKgel Octadecyl-4PW column (0.46 cm × 15 cm; Tosoh, Tokyo, Japan) equilibrated with 0.1% trifluoroacetic acid (TFA). The column was eluted at a flow rate of 1 mL/min by a gradient of acetonitrile (see Fig. 1c for details) in 0.1% TFA.

The major allergen (Tur c 1) of the turban shell was purified as described elsewhere¹⁵ and used as a reference in some experiments.

Protein determination

Protein was determined by the method of Lowry *et al.*,¹⁸ using bovine serum albumin as a standard.

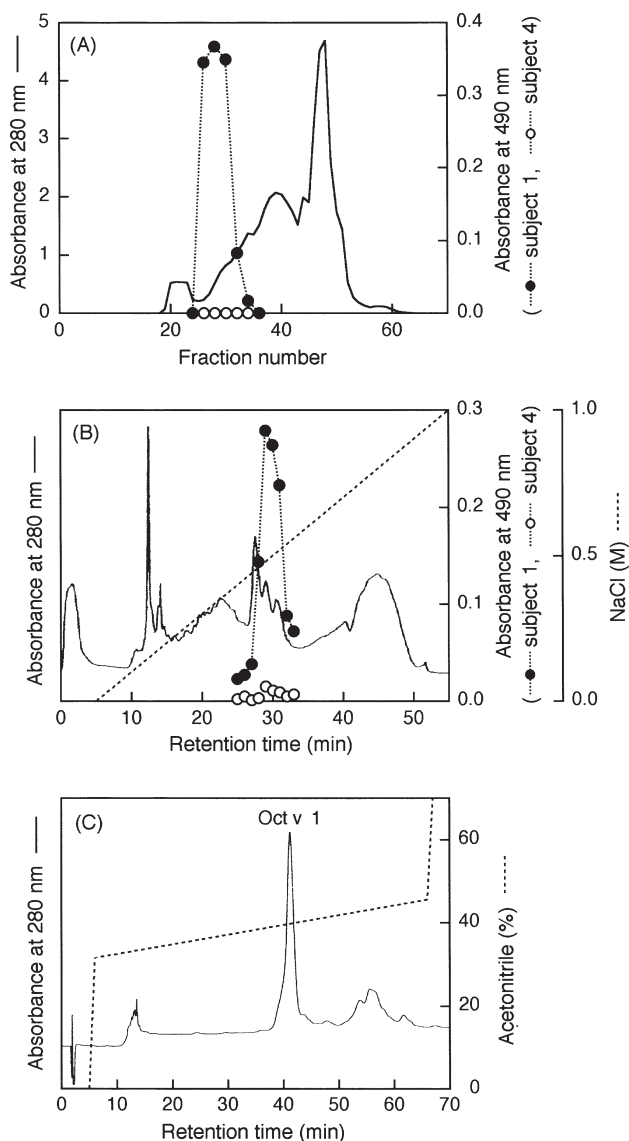


Fig. 1 Purification of the octopus allergen (Oct v 1) by (a) gel filtration on Sephacryl S-200; (b) cation-exchange fast protein liquid chromatography on Mono Q; and (c) reverse-phase high-pressure liquid chromatography on TSKgel Octadecyl-4PW. Subject 1 serum was used as a sensitized serum and subject 4 serum as a control.

SDS-PAGE and immunoblotting

Sodium dodecylsulfate–polyacrylamide gel electrophoresis was performed on a PhastSystem apparatus (Pharmacia) using ready-made slab gels (PhastGel Gradient 10-15) and ready-made buffer strips (PhastGel SDS Buffer Strips). Running and staining of the gel with Coomassie Brilliant Blue R-250 were carried out according to the manufacturer's instruction. Prior to SDS-PAGE, each sample was dissolved in 0.01 M Tris-HCl buffer (pH 6.8)

containing 1% SDS, 20% glycerin and 5% dithiothreitol and heated in a boiling water bath for 10 min. A low molecular weight electrophoresis calibration kit (Pharmacia), containing phosphor-lyase b (94 k), bovine serum albumin (67 k), ovalbumin (43 k), carbonic anhydrase (30 k), soybean trypsin inhibitor (20.1 k) and α -lactalbumin (14.4 k), was used as a reference.

Immunoblotting was also carried out on the PhastSystem apparatus according to the manufacturer's instruction. The proteins separated by SDS-PAGE were electroblotted from the gel to a nitrocellulose membrane. The membrane was blocked with 5% skimmed milk in Dulbecco's phosphate-buffered saline (blocking solution) at room temperature for 24 h and incubated with subject 1 serum (diluted 1:25 with blocking solution) for 20 h. After washing with 0.05% Tween-20 in Dulbecco's phosphate-buffered saline, the membrane was incubated with peroxidase-conjugated goat antihuman IgE antibody (diluted 1:5000 with blocking solution) for 1 h. Antigen-antibody binding was visualized by the addition of substrate solution containing 0.05% 3,3'-diaminobenzidine tetrahydrochloride (Sigma Chemical Co.) and 0.03% H_2O_2 .

Amino acid analysis

The purified octopus allergen (50 μ g) was hydrolyzed with 80 μ L of 4 M methanesulfonic acid containing 0.2% 3-(2-aminoethyl) indole in an evacuated tube at 115°C for 24 h. After hydrolysis, the solution was neutralized with an equal volume of 3.5 M NaOH and diluted with 800 μ L of 0.2 M citrate buffer (pH 2.2). A 400- μ L portion of the dilution was applied to an MLC-703 amino acid analyzer (Atto, Tokyo, Japan).

Reduction and S-carboxamidomethylation

Purified octopus allergen (300 μ g) was dissolved in 300 μ L of 0.5 M Tris-HCl buffer (pH 8.5) containing 7 M guanidine-HCl and 0.01 M EDTA and reduced with 0.6 mg of dithiothreitol under nitrogen gas at room temperature for 2 h. Then, 1.5 mg of iodoacetamide was added and the reaction mixture was kept in the dark at room temperature for 30 min. The S-carboxamidomethylated (CAM) derivative produced was isolated by reverse-phase HPLC on a TSKgel Octadecyl-4PW column (0.46 cm $\times$ 15 cm). Elution of the column was performed at a flow rate of 1 mL/min by a linear gradient of acetonitrile (0–70% in 100 min) in 0.1% TFA. The CAM allergen was monitored at 280 nm with a UV detector.

Enzymatic digestion

The CAM allergen (250 µg) was digested with 1 µg of lysylendopeptidase (E.C. 3.4.21.50; Wako, Osaka, Japan) in 500 µL of 0.1 M Tris-HCl buffer (pH 9.0) at 35°C for 6 h. In order to separate peptide fragments, the digest was applied to reverse-phase HPLC on a TSKgel ODS-120T column (0.46 cm × 25 cm; Tosoh), which was eluted at a flow rate of 1 mL/min by a linear gradient of acetonitrile (0–56% in 120 min) in 0.1% TFA. Peptides were monitored at 220 nm with a UV detector. Each peptide was semiquantified by comparing the peak area with that of the purified allergen used as a standard.

Analysis of amino acid sequence

Sequence analyses were performed by the automated Edman degradation method using a gas-phase protein sequencer (LF-3400D TriCart with high-sensitivity chemistry; Beckman Coulter, Fullerton, CA, USA).

RESULTS

Purification of the octopus allergen

When the crude extract of the octopus muscle was heated, no decrease in the absorbance value of ELISA was observed, suggesting that the major allergen was heat-stable. In gel filtration on Sephacryl S-300, the major allergen was eluted immediately after the void volume with a significant reactivity only to the sensitized serum (Fig. 1a). Allergen-containing fractions (fractions 26–32) were combined and subjected to a Mono Q HR 5/5 column. The major allergen was absorbed by the column and eluted at a NaCl concentration of approximately 0.5 M (Fig. 1b). The eluate corresponding to the retention times of 27–31 min was applied to the final step of purification by reverse-phase HPLC on TSKgel Octadecyl-4PW, in which the major allergen was eluted in a peak at a retention time of 41 min (Fig. 1c). The purified allergen thus obtained was demonstrated to be tropomyosin as described below and hence designated as Oct v 1, in accordance with the guidelines proposed by the International Union of Immunological Societies allergen nomenclature regulation.

Properties of the purified allergen

In SDS-PAGE, Oct v 1 gave three bands in the range of 31–34 kDa (Fig. 2a). Immunoblotting proved that

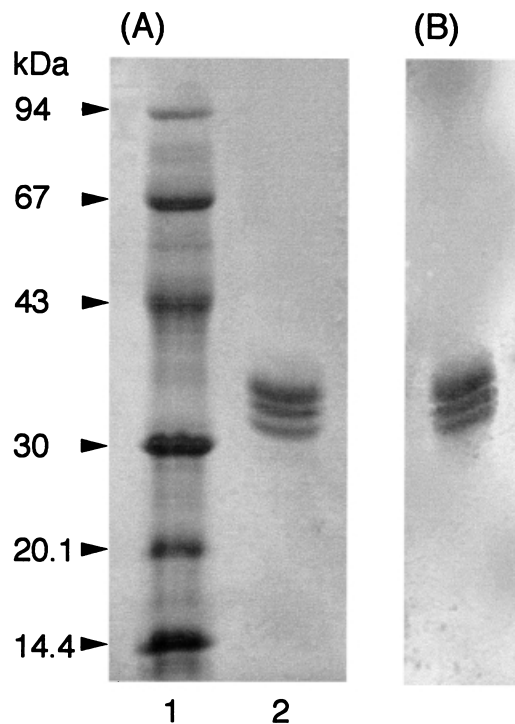


Fig. 2 (a) Sodium dodecylsulfate–polyacrylamide gel electrophoresis and (b) immunoblotting of Oct v 1. (a) Lane 1, standard proteins; lane 2, Oct v 1. Proteins were stained with Coomassie brilliant blue R-250. (b) Oct v 1 was detected by enzyme-linked immunosorbent assay using the subject 1 serum.

all of these bands electrotransferred to a membrane had immunoreacted with IgE antibodies in the sensitized serum (Fig. 2b). As listed in Table 1, the amino acid composition of Oct v 1 was characterized by the abundance of Glx, followed by Asx, Leu, Ala and Lys in decreasing order of abundance, and also by the absence of Pro and Met. Analyses by ELISA revealed that Oct v 1 was commonly allergic to subjects 1–3. All the three sensitized sera reacted to Oct v 1 in a dose-dependent manner, although the reactivity of subject 2 serum was considerably higher than that of the other two sera (Fig. 3). A similar but somewhat higher IgE reactivity of the sera was also observed against Tur c 1, the turban shell allergen of 35 kDa that was used as a reference. In competitive ELISA inhibition experiments, the reactivity between the sensitized sera and Oct v 1 was reduced markedly not only by Oct v 1 but also by Tur c 1, demonstrating cross-reactivity between both allergens (Fig. 4). Higher reducing effects recognized with Tur c 1 were consistent with the fact that the sensitized sera are more reactive to Tur c 1 than Oct v 1.

Partial amino acid sequence of the purified allergen

Because Oct v 1 was shown to have 0.4% half-Cys (Table 1), its CAM derivative was prepared prior to enzymatic digestion and isolated by reverse-phase HPLC (data not shown). Twenty peptides (named K1–20 in the order of elution) were separated from the lysylendopeptidase digest of CAM–Oct v 1 by reverse-phase HPLC (Fig. 5) and the six peptides selected (K7, K10, K11, K18, K19 and K20) were subjected to sequence analyses. The determined partial amino acid sequence of Oct v 1 is shown in Fig. 6, along with the reported partial or complete

sequences of tropomyosins from the squid *T. pacificus* (Tod p 1),¹⁶ the oyster *C. gigas* (Cra g 1),¹⁴ the turban shell *T. cornutus* (Tur c 1),¹⁵ the abalone *Haliotis rufescens* and two species of shrimp, *P. indicus* (Pen i 1)⁷ and *P. aztecus* (Pen a 1).¹⁷ A comparison of the sequences in the overlapped regions showed that Oct v 1 had high homologies (57–97%) with the tropomyosins from other sources. In particular, there was only one replacement (at position 49) in the overlapped 30 residues between Oct v 1 and Tod p 1 from the squid, which is a member of the cephalopods as is the octopus. Oct v 1 was thus identified as the tropomyosin of *O. vulgaris*.

Immunoglobulin E-binding epitopes of the purified allergen

Figure 7 shows the IgE reactivities of the 20 peptides generated by lysylendopeptidase digestion of CAM–Oct v 1, which were evaluated indirectly by the competitive ELISA inhibition method. In the case of subject 1 serum, peptide 19 inhibited the reactivity between Oct v 1 and serum IgE antibodies most effectively. Peptides 18 and 20 also displayed considerably high inhibition. Similar results were obtained with subject 3 serum, except that an additional two peptides (K12 and K13) were inhibitory comparable to peptides 18 and 20. These results led us to assume that three peptides (K18, K19 and K20) are commonly reactive to IgE against the sera from subjects 1 and 3; that is, they contain IgE-binding epitopes of Oct v 1. In contrast, no significant inhibition was recognized in any of the 20 peptides when tested using subject 2 serum, suggesting that the epitope structures of Oct v 1 against this serum differed from those against the other two sera.

Table 1 Amino acid composition of Oct v 1

Amino acid	Mol %
Asx	14.1
Thr	4.2
Ser	3.6
Glx	24.1
Pro	0.0
Gly	2.9
Ala	11.4
Val	4.1
Cys (half)	0.4
Met	0.0
Ile	3.3
Leu	12.2
Tyr	0.4
Phe	1.1
His	0.5
Trp	0.1
Lys	9.8
Arg	7.9

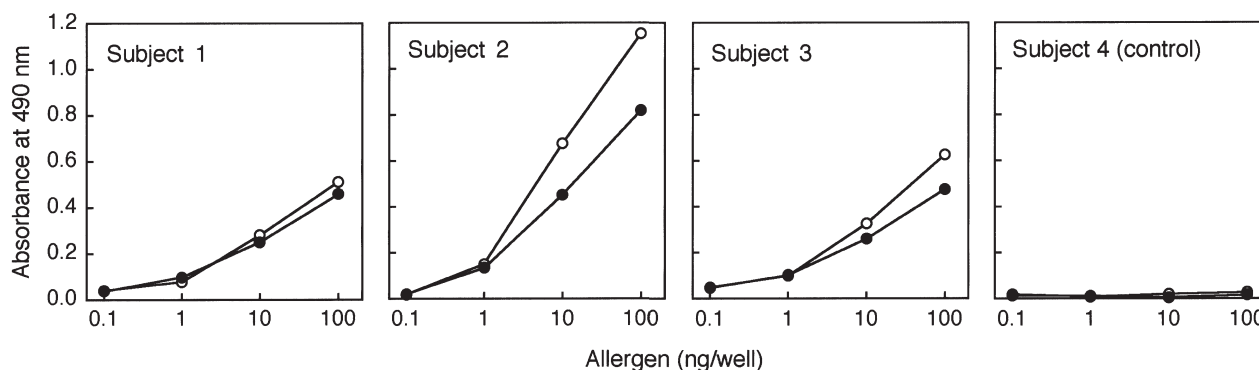


Fig. 3 Analysis by enzyme-linked immunosorbent assay of the immunoglobulin E reactivity of patient (subjects 1–3) and control (subject 4) sera to (●) Oct v 1 and (○) Tur c 1.

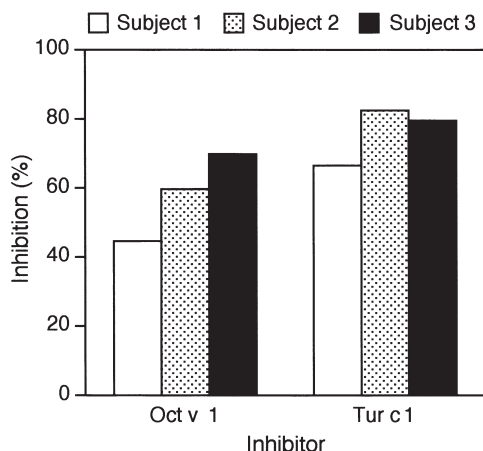


Fig. 4 Inhibition of the immunoglobulin E reactivity of patient (subjects 1–3) sera to Oct v 1 by Oct v 1 and Tur c 1. Each percentage inhibition was calculated using the following equation: % Inhibition = $(A - B) \times 100 / A$, in which A and B represent the reactivities (expressed as absorbance at 490 nm) of patient serum to Oct v 1 in the absence and presence of inhibitor (Oct v 1 or Tur c 1), respectively.

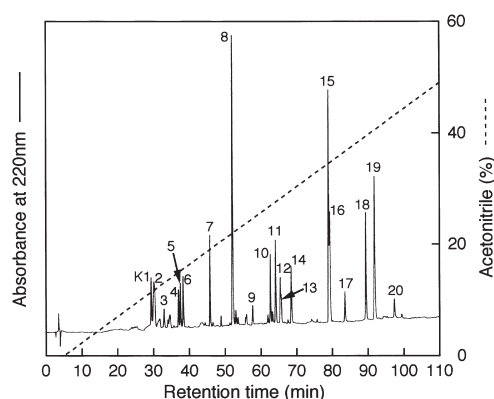


Fig. 5 Isolation of peptide fragments from the lysylendopeptidase digest of S-carboxamidomethylated (CAM)-Oct v 1 by reverse-phase high-pressure liquid chromatography on TSKgel ODS-120T. Twenty peptides (K1–20) were isolated.

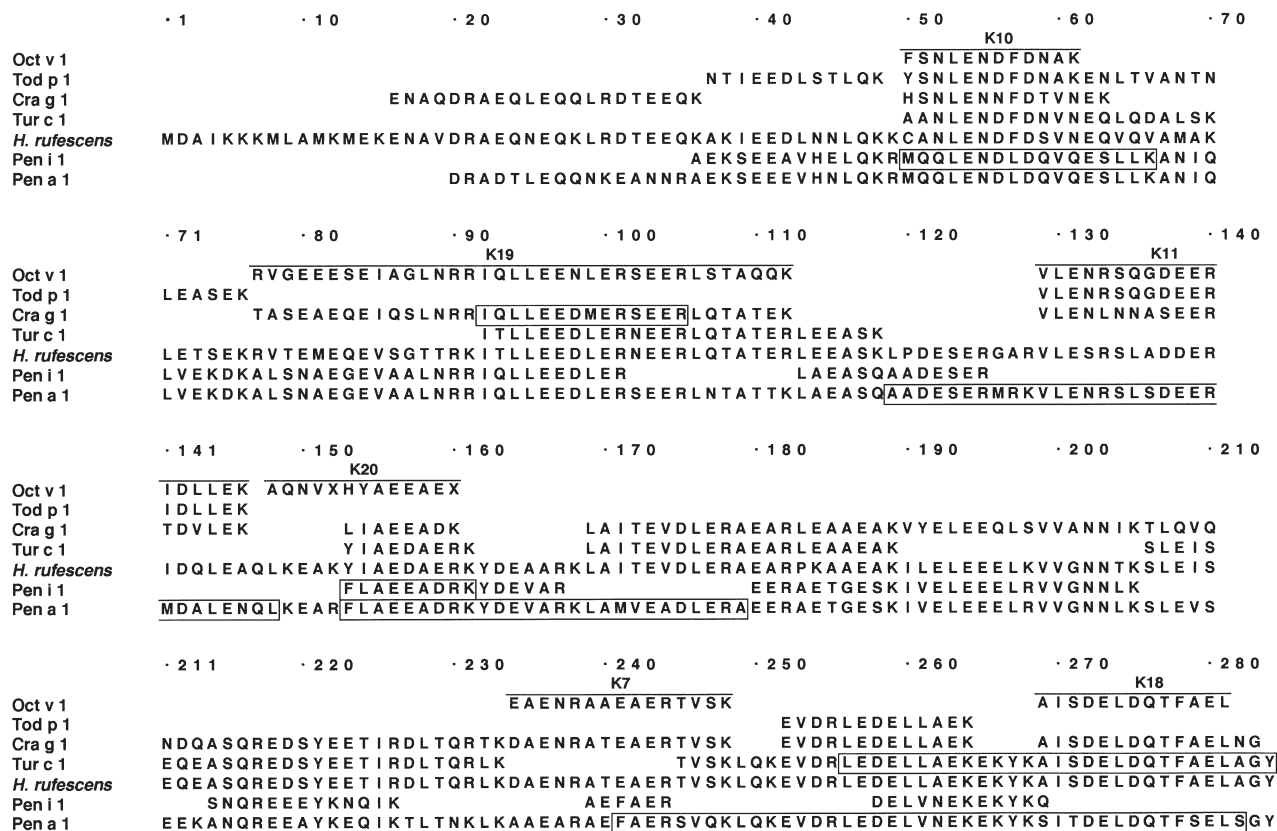


Fig. 6 Alignment of the determined partial amino acid sequence of Oct v 1 with the reported partial or complete sequences of Tod p 1, Cra g 1, Tur c 1, tropomyosin from the abalone *Haliotis rufescens*, Pen i 1 and Pen a 1. Numbering is based on the sequence of the *H. rufescens* tropomyosin, which was translated from the nucleotide sequence database (EMBL accession no. X75218). In the case of Oct v 1, the denotations preceded with the letter K represent the peptides isolated from the lysylendopeptidase digest (see Fig. 5). Unidentified residues at positions 152 and 160 (in peptide K20) are indicated by the letter X. The proposed IgE-binding epitopes for Cra g 1, Tur c 1, Pen i 1 and Pen a 1 are boxed.

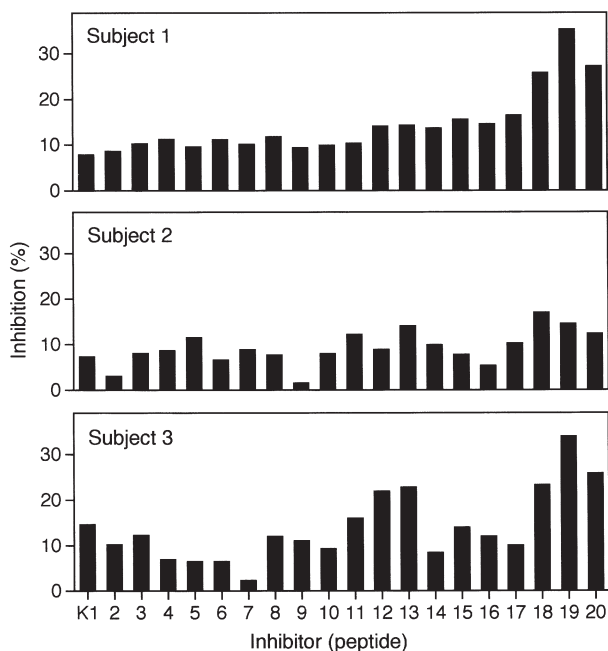


Fig. 7 Inhibition of the immunoglobulin E reactivity of patient (subjects 1–3) sera to Oct v 1 by peptides K1–20 isolated from the lysylendopeptidase digest of *S*-carboxamidomethylated (CAM)–Oct v 1. Each percentage inhibition was calculated using the following equation: % Inhibition = $(A - B) \times 100 / A$, in which A and B represent the reactivities (expressed as absorbance at 490 nm) of patient serum to Oct v 1 in the absence and presence of inhibitor (peptide), respectively.

DISCUSSION

The major allergen (Oct v 1) in the muscle of the octopus *O. vulgaris* was purified essentially using the same method adopted for the allergens of oyster¹³ and turban shell,¹⁵ except that a TSKgel Phenyl-5PW was replaced by a TSKgel Octadecyl-4PW during reverse-phase HPLC. In addition to the amino acid composition and the cross-reactivity with Tur c 1, the determined partial amino acid sequence clearly demonstrated Oct v 1 to be tropomyosin. However, the observation of three adjacent bands in SDS-PAGE (Fig. 2a) may be rather unusual for tropomyosin, being ascribable to the partial degradation of tropomyosin during purification or to the presence of its isomeric forms. Apart from this unusual result, which requires further clarification, the results of the present study, together with those for the squid *T. pacificus*,¹⁶ strongly suggest that the major allergen in cephalopods is tropomyosin, similar to the known molluscan and crustacean allergens.

Competitive ELISA inhibition experiments,

using peptide fragments derived from CAM–Oct v 1, proved that IgE-binding epitopes of Oct v 1 differ from serum to serum. In the present study, no IgE-reactive peptides were found against subject 2 serum, suggesting that the epitope structures of Oct v 1 against this serum were destroyed to a great extent by lysylendopeptidase. It is, however, apparent that IgE-binding epitopes against the sera from subjects 1 and 3 are commonly contained in at least three peptides (K18, K19 and K20). Immunoglobulin E-binding epitopes of four molluscan and crustacean allergens, Cra g 1 from the oyster *C. gigas*,^{14,19} Tur c 1 from the turban shell *T. cornutus*,¹⁵ Pen i 1 from the shrimp *P. indicus*⁷ and Pen a 1 from the shrimp *P. aztecus*,¹⁷ have recently been proposed as follows: segment 92–105 for Cra g 1; segment 256–284 for Tur c 1; segments 50–66 and 153–161 for Pen i 1; and segments 119–148, 153–179 and 241–282 for Pen a 1 (see Fig. 6). Some of these proposed IgE-binding epitopes are related to those for Oct v 1. Therefore, the three IgE-binding epitopes of Oct v 1 are individually compared in detail with those of the four allergens, as described below.

The peptide K18 (positions 269–281) is located in the C-terminal region and its corresponding segment is included in the IgE-binding epitope (256–284) of Tur c 1 and one (241–282) of Pen a 1. When compared with the corresponding region of Pen a 1, the peptide K18 has three alterations at positions 269, 271 and 279, but these alterations do not seem to affect its binding ability to specific IgE antibodies in sensitized sera. Alternatively, there are no alterations between the peptide K18 and the corresponding region of Tur c 1. The same sequence as the peptide K18 is also recognized in peptide 269–283 derived from Cra g 1. However, using competitive ELISA inhibition experiments, this peptide has been evaluated previously as not being significantly reactive to IgE probably because of the differences in sera used.¹⁴

The peptide K19 (positions 77–112), which is considered to be the most reactive to IgE based on the results of competitive ELISA inhibition experiments (Fig. 7), includes the whole segment corresponding to the IgE-binding epitope (92–105) of Cra g 1. A previous study using synthetic peptides demonstrated that both N-terminal and C-terminal regions (⁹²IQLL⁹⁶ and ¹⁰³EER¹⁰⁵) in the IgE-binding epitope of Cra g 1 are more essential for binding to IgE antibodies in sensitized sera than the middle region (⁹⁷EDMERS¹⁰²).¹⁹ A comparison of the sequences of Cra g 1 shows two alterations (Asp to Asn at position 98 and Met to Leu at position 99) in the peptide K19. However, the sequences at positions 92–96 and 103–105 are well conserved, supporting the IgE-binding ability of

the peptide K19. In the case of Tur c 1, peptide 92–118 is not regarded as a candidate epitope for IgE-binding.¹⁵ This appears to be related greatly to the replacement of Gln by Thr at position 93, which is an important position for IgE reactivity.

As for the peptide K20 (positions 148–160), partial overlapping with the IgE-binding epitopes of shrimp allergens (153–161 for Pen i 1 and 153–179 for Pen a 1) is recognized. According to Shanti *et al.*, who studied Pen i 1, two residues, Phe-153 and Leu-154, are critical for binding to specific IgE antibodies.⁷ Although alterations at positions 153 and 154 are observed throughout all of the peptide K20, positions 153–160 from Cra g 1 and positions 153–161 from Tur c 1, only the peptide K20 is significantly reactive to IgE. Further studies using various synthetic peptides would provide a better understanding of the relationship between the residues at positions 153 and 154 and IgE reactivity.

In summary, the IgE-binding epitopes for Oct v 1 are associated with some of those reported for molluscan allergens (Cra g 1 and Tur c 1) and shrimp allergens (Pen i 1 and Pen a 1). Interestingly, in the present study, the segment (K20) corresponding to one of the IgE-binding epitopes of Pen i 1 was found to be reactive to IgE, differing from the case of the two molluscan allergens. However, it should be emphasized that the results concerning the IgE-binding epitopes of tropomyosins, including the results of the present study, are still incomplete. For example, Oct v 1 still has unknown IgE-binding epitopes against some sensitized sera such as the subject 2 serum. Although the peptides K10 (positions 50–61) and K11 (positions 129–146) partly overlap with one of the IgE-binding epitopes of Pen i 1 and that of Pen a 1, respectively, it is unclear whether their inability to bind to IgE antibodies is because of a lack of critical amino acid residues or because of the diverse replacements of critical residues. In addition, quite different results have been reported even for Pen i 1 and Pen a 1, allergens found in closely related shrimps, although their amino acid sequences are highly homologous (97% homology) with each other. The segment 50–66 has been identified as one of the IgE-binding epitopes for Pen i 1 by Shanti *et al.*⁷ but not for Pen a 1 by Reese *et al.*¹⁷ On the contrary, comments on the segment 258–269 were not given for Pen i 1 by Shanti *et al.*, but its elongated segment (241–282) was evaluated as one of the IgE-binding epitopes for Pen a 1 by Reese *et al.* In view of these circumstances, further study, using many sensitized sera or pooled serum from many patients, is needed to obtain more detailed information about IgE-binding epitopes of molluscan and crustacean tropomyosins, including Oct v 1.

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