



Purification, reactivity with IgE and cDNA cloning of parvalbumin as the major allergen of mackerels

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Abstract

Three species of mackerels (*Scomber japonicus*, *S. australasicus* and *S. scombrus*) are widely consumed and considered to be most frequently involved in incidents of IgE-mediated fish allergy in Japan. In this study, parvalbumin, a possible candidate for the major allergen, was purified from the white muscle of three species of mackerels by gel filtration on Sephadex G-75 and reverse-phase HPLC on TSKgel ODS-120T. All the purified preparations from three species gave a single band of about 11 kDa and were clearly identified as parvalbumins by analyses of their partial amino acid sequences. In ELISA experiments, four of five sera from fish-allergic patients reacted to all the purified parvalbumins, demonstrating that parvalbumin is the major allergen in common with the mackerels. Antigenic cross-reactivity among the mackerel parvalbumins was also established by ELISA inhibition experiments. A cDNA library was constructed from the white muscle of *S. japonicus* and the cDNA encoding parvalbumin was cloned. The amino acid sequence translated from the nucleotide sequence revealed that the *S. japonicus* parvalbumin is composed of 108 residues, being a member of β -type parvalbumins.

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1. Introduction

Fish is among the most common causes of food allergy mediated by IgE antibodies, especially in coastal countries such as Scandinavia and Japan where fish consumption is high. After ingestion of fish, some of the following clinical symptoms are rapidly induced in sensitized patients: urticaria, angioedema, respiratory symptoms (asthma and rhinoconjunctivitis), gastrointestinal symptoms (diarrhea and vomiting) and, in severe cases, fatal anaphylaxis (Pascual et al., 1992; O'Neil C et al., 1993). Extensive studies with cod *Gadus callarias* first identified parvalbumin (known as Gad c 1), a calcium-binding sarcoplasmic protein with a molecular mass of about 12 kDa, as a fish allergen

(Elsayed and Aas, 1971a; Elsayed and Bennich, 1975). Subsequent molecular studies have established that the major fish allergen is parvalbumin (Lindström et al., 1996; Shiomi et al., 1998, 1999; Bugajska-Schretter et al., 1999; Swoboda et al., 2002), although minor allergens have been simultaneously detected in various species of fish by immunoblotting (Bernhisel-Broadbent et al., 1992a; Pascual et al., 1992; Hansen et al., 1997; James et al., 1997) and one of them recently purified and identified as collagen (Hamada et al., 2001).

Allergenicity of fish parvalbumins is reduced to some extent when their bound calcium is released (Swoboda et al., 2002) but is very stable to heating, extreme pH and treatment with denaturing agents (Elsayed and Aas, 1971b). This fact suggests that allergenic activity of parvalbumins is dependent mainly on the amino acid sequence, although the contribution of their steric conformation is not excluded. Indeed, at least five segments of Gad c 1 have already been proposed as IgE-binding (B-cell) epitopes (Elsayed and Apold, 1983). Therefore, in order to reveal the molecular basis relating to the allergenicity of fish parvalbumins, it is essential to determine their complete amino acid sequences. For this

Abbreviations: BSA, bovine serum albumin; DPBS, Dulbecco's phosphate buffered saline; ELISA, enzyme-linked immunosorbent assay; HPLC, high performance liquid chromatography; IPTG, isopropyl-thio- β -D-galactoside; SDS-PAGE, sodium dodecylsulfate-polyacrylamide gel electrophoresis; TFA, trifluoroacetic acid.

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purpose, a cDNA cloning technique is very useful. Following the elucidation of T-cell and IgE-binding epitopes, the isolated cDNAs encoding fish parvalbumins can be also used to develop hypoallergenic molecules for specific immunotherapy of fish allergy. So far, parvalbumins of only two species of fish, Atlantic salmon (Lindström et al., 1996) and carp (Swoboda et al., 2002), have been cloned for their cDNAs from the viewpoint of fish allergy.

Three species of mackerels (Pacific mackerel *Scomber japonicus*, spotted mackerel *S. australasicus* and Atlantic mackerel *S. scombrus*) are widely circulated and consumed in Japan. They are considered to be most frequently responsible for fish allergy in Japan and hence processed food products containing them as raw materials are recommended to put labels indicating their use on packages or bottles. At present, however, no information is available about whether three species of mackerels can be assessed to be equally allergenic. In addition, parvalbumin is the most possible candidate for the major allergen in three species of mackerels, as suggested by previous immunoblotting studies (Hansen et al., 1997), but no molecular evidence has been obtained. In this study, therefore, parvalbumins were purified from three species of mackerels and their reactivities with IgE in fish-allergic patients were compared to one another. For the convenience of future molecular study, such as elucidation of epitopes, cDNAs encoding the *S. japonicus* parvalbumin were also cloned.

2. Materials and methods

2.1. Fish

Specimens of *S. japonicus* and *S. australasicus* were captured along the coasts of Kanagawa and Ohita Prefectures, respectively, and shipped alive to our laboratory. Specimens of *S. scombrus* imported from Norway were purchased at a retail store in Tokyo. Aliquots of the white muscle were obtained from one live specimen of *S. japonicus* for cDNA cloning experiments and kept at -80°C until use. The remaining specimens of the three species were stored at -20°C until use.

2.2. Human sera

Sera were obtained from five fish-allergic patients (Table 1), who had a documented clinical history of immediate hypersensitivity reactions after ingestion of fish. The five patients had been shown to have elevated serum IgE specific for fish by CAP-RAST done at hospitals. In this study, sera from two healthy subjects without adverse reactions after ingestion of any foods were used as controls. All sera were divided into portions and stored at -20°C until use.

2.3. ELISA

Enzyme-linked immunosorbent assay (ELISA) was performed using a flat-bottomed polystyrene plate with 96 wells (Type H Multi Well Plate for ELISA; Sumitomo Bakelite, Tokyo, Japan), essentially as reported previously (Ishikawa et al., 1997). In brief, each sample coated on the plate was reacted with patient or control serum, diluted 1:50 with 0.1% bovine serum albumin (BSA) in Dulbecco's phosphate-buffered saline (pH 7.4, DPBS), followed by peroxidase-conjugated goat anti-human IgE antibody (diluted 1:2,500 with 0.1% BSA in DPBS; Kirkegaard & Perry Laboratories, Gaithersburg, MD). Enzyme reaction was carried out using substrate solution containing 0.1% *o*-phenylenediamine and 0.03% H_2O_2 and arrested by addition of 1 M sulfuric acid. The developed color was measured by absorbance at 490 nm. In order to detect parvalbumin in chromatographic steps, the serum was replaced by the mouse anti-frog muscle parvalbumin monoclonal antibody (diluted 1:5,000 with 0.1% BSA in DPBS; Sigma, St. Louis, MO) and peroxidase-conjugated goat anti-human IgE antibody by peroxidase-conjugated goat anti-mouse IgG antibody (diluted 1:5000 with 0.1% BSA in DPBS; Kirkegaard & Perry Laboratories).

For ELISA inhibition experiments, each patient serum was diluted 1:25 with 0.1% BSA in DPBS and incubated at 37°C for 1 h with an equal volume of mackerel parvalbumin solution (10 $\mu\text{g}/\text{ml}$) made in DPBS. A 100 μl portion of this solution was then added to a microtiter plate that had previously been coated with the *S. japonicus* parvalbumin at a concentration of

Table 1
List of fish-allergic patients

Patient	Age	Gender	CAP-RAST class	Clinical symptoms after fish ingestion
1	1	Female	Salmon (3), cod (3)	Urticaria
2	4	Female	Salmon (4), cod (3)	Urticaria
3	8	Male	Salmon (3), cod (3), horse mackerel (3), mackerel (3)	Urticaria, asthma
4	16	Male	Sardine (3), salmon (3), cod (3), horse mackerel (3), mackerel (3), tuna (3)	Urticaria
5	19	Male	Cod (4), horse mackerel (3), mackerel (3), tuna (4)	Urticaria, diarrhea

0.1 µg/well. The subsequent procedure was the same as that for the ELISA described above.

All ELISAs were performed in triplicate and the data were expressed as the mean values.

2.4. Protein determination

Proteins were determined by the method of Lowry et al. (1951), using BSA as a standard.

2.5. Purification of parvalbumin

The white muscle was extracted with three volumes of 0.15 M NaCl in 0.01 M phosphate buffer (pH 7.0) in a boiling water bath for 10 min. The heated extract was subjected to gel filtration on a Sephadex G-75 column (2.5×98 cm; Amersham Pharmacia Biotech, Buckinghamshire, UK), which was eluted with 0.15 M NaCl in 0.01 M phosphate buffer (pH 7.0). Fractions of 10 ml were collected, measured for absorbance at 280 nm and assayed for parvalbumin by ELISA. Parvalbumin-containing fractions were pooled and then applied to reverse-phase HPLC on a TSKgel ODS-120T column (0.46×25 cm; Tosoh, Tokyo, Japan). The column was eluted first with 0.1% trifluoroacetic acid (TFA) for 5 min, followed by two steps of linear gradients of acetonitrile (0–35% in 2 min and 35–49% in 60 min) in 0.1% TFA, at a flow rate of 1 ml/min. Proteins were monitored at 220 nm with a UV detector. The eluate judged to contain parvalbumin by ELISA was manually collected, lyophilized and dissolved in desired solvents for subsequent experiments.

2.6. Electrophoresis

Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a PhastSystem (Amersham Pharmacia Biotech) was carried out according to the method described in the manufacturer's instructions. Ready-made gels (PhastGel High Density), ready-made buffer strips (PhastGel SDS Buffer Strips) and a molecular weight calibration kit (containing five proteins or peptides of 2512–16,949 Da) were purchased from Amersham Pharmacia Biotech. Prior to running, each sample was treated with 2.5% SDS containing 5% dithiothreitol at 100 °C for 10 min. Proteins were visualized by staining with Coomassie Brilliant Blue R-250.

2.7. Enzyme digestion and isolation of peptide fragments

Each purified mackerel parvalbumin (100 µg) was digested with 2 µg of lysylendopeptidase (E.C.3.4.21.50; Wako Pure Chemicals, Osaka, Japan) in 0.3 ml of 0.025 M Tris–HCl buffer (pH 8.5) containing 1 mM EDTA at 35 °C for 24 h. In order to separate peptide fragments, the digest was applied to reverse-phase HPLC on a

TSKgel ODS-120T column (0.46×25 cm; Tosoh), which was eluted at a flow rate of 1 ml/min by a linear gradient of acetonitrile (0–50% in 120 min) in 0.1% TFA. Peptides were monitored at 220 nm with a UV detector.

2.8. Amino acid sequencing

Amino acid 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).

2.9. Construction of cDNA library

Total RNA was extracted from 2 g of the white muscle of *S. japonicus* by the guanidium isothiocyanate method using the TRIZol reagent (Life Technologies, Gaithersburg, MD) and poly(A)⁺ mRNA was purified by affinity chromatography on oligo(dT)-cellulose using an mRNA purification kit (Amersham Pharmacia Biotech). Synthesis of cDNA and ligation of the double-stranded cDNA to *Not* I/*Sal* I ends of λZipLox were carried out using the SuperScriptTM Lambda System (Life Technologies) and packaging in λPhage using the Gigapack III Gold Packaging Extract (Stratagene, La Jolla, CA).

2.10. Screening of cDNA library

The cDNA library constructed was infected to *Escherichia coli* Y1090 and plated on LB agar at a density of 3×10⁴/plate. After incubation at 42 °C for 3.5 h, plaques formed were blotted onto a nitrocellulose membrane, which had previously been immersed in 10 mM isopropyl-thio-β-D-galactoside (IPTG), at 37 °C for 4–8 h. The membrane was blocked with blocking solution, 3% BSA in 0.05 M Tris–HCl buffer (pH 8.0) containing 0.15 M NaCl and 0.05% Tween 20 (TBS-Tween), at 4 °C overnight and reacted with the anti-frog muscle parvalbumin monoclonal antibody (diluted 1:500 with blocking solution) at room temperature for 2 h. After being washed three times with 0.1% BSA in TBS-Tween, the membrane was incubated with peroxidase-conjugated goat anti-mouse IgG antibody (diluted 1:5000 with blocking solution) at room temperature for 1 h. Labeled proteins were visualized by enzyme reaction using substrate solution containing 0.048% 4-chloro-1-naphthol and 0.015% H₂O₂.

2.11. DNA sequencing

Positive λZipLox clones were infected to *E. coli* DH 10B, in which the plasmid pZL1 was excised from λ phage, and subjected to blue-white selection on LB agar containing 0.01% ampicillin, 0.00625% X-gal and 2 mM IPTG. The plasmid was purified from *E. coli* DH 10B, which had been picked up from a white colony and

grown in LB medium containing 0.01% ampicillin, using a Quantum Prep Plasmid Miniprep kit (Bio-Rad Laboratories, Hercules, CA). Cycle sequencing of the plasmid DNA with Ampli Taq DNA polymerase and dye-labeled terminators was carried out using an ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction kit (Perkin-Elmer, Foster City, CA) and sequencing primers (M13/pUC 23-base forward primer and T7 promoter reverse primer). Nucleotide sequences were determined on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA).

3. Results and discussion

3.1. Purification of parvalbumins from three species of mackerels

In the case of *S. japonicus*, proteins showing reactivity with the anti-frog muscle parvalbumin monoclonal antibody were eluted at around fraction 38 from a Sephadex G-75 column (Fig. 1A). Almost the same chromatograms were also observed in both *S. australasicus* and *S. scombrus* (data not shown). Positive fractions were pooled and then subjected to reverse-phase HPLC on TSKgel ODS-120T. Regardless of the species, proteins positive to the anti-frog muscle parvalbumin monoclonal antibody appeared in a single peak with a retention time between 40 and 50 min (Fig. 1B). In SDS-PAGE, each purified preparation migrated as a single band at a position corresponding to about 11 kDa, supporting the homogeneity (Fig. 2).

None of the purified preparations afforded any N-terminal amino acid residue by direct sequencing, indicating the blocking of their N-terminus, as previously recognized in fish parvalbumins (Elsayed et al., 1973; Lindström et al., 1996). In order to determine the par-

tial amino acid sequences of the purified preparations, therefore, they were individually digested with lysylendopeptidase and some of the peptide fragments, which were isolated by reverse-phase HPLC on TSKgel ODS-120T, were sequenced (Fig. 3). As compared to the entire amino acid sequence of the *S. japonicus* parvalbumin deduced from the nucleotide sequence (refer to Fig. 7), which is detailed later, the four fragments (AFAIDQDK, SGFIEEEELK, LFLQNFK and IGI-DEFAAMIK) from the *S. japonicus* preparation have the same sequences as the segments 46–54, 55–64, 65–71 and 97–107, respectively, and the four fragments (DAEVTAALDGCK, AAGSFDHK, ACGLSGK and IGIDEFAAMIK) from the *S. australasicus* preparation as the segments 8–19, 20–27, 32–38 and 97–107, respectively. In the case of *S. scombrus*, one fragment (AAGSFDHK) is identical with the segment 20–27 of the *S. japonicus* parvalbumin, while the other two fragments (SGYIEEEELK and IGVDEFAAK) have one or two alterations but well correspond to the segments 55–64 and 97–105, respectively. These results confirmed that the purified preparations from three species of mackerels are none other than parvalbumins.

3.2. Reactivity of mackerel parvalbumins with IgE in fish-allergic patients

When analyzed by ELISA, four of the five patient sera tested reacted to all the purified mackerel parvalbumins (Fig. 4). Although future study using more patient sera and in vivo tests such as a skin prick test may be needed, the results strongly suggest that parvalbumin is the major allergen in common with three species of mackerels, as already established at the molecular level in several species of fish (Elsayed and Aas, 1971a; Elsayed and Bennich, 1975; Lindström et al., 1996; Shiomi et al., 1998, 1999; Bugajsk-Schretter et

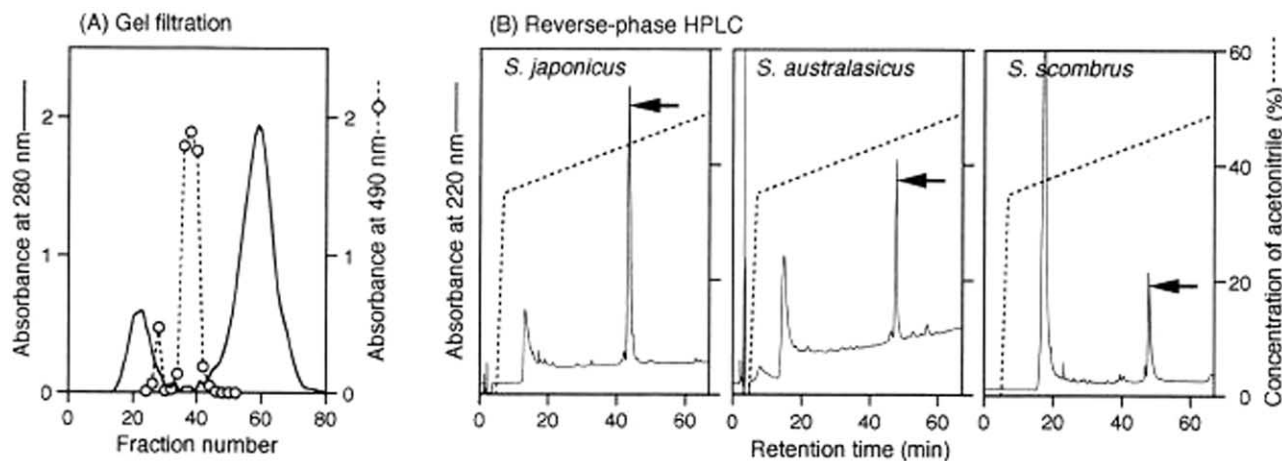


Fig. 1. Purification of mackerel parvalbumins: (A) gel filtration on Sephadex G-75 of the crude extract from *S. japonicus*; (B) reverse-phase HPLC on TSKgel ODS-120T. Parvalbumin-containing fractions from *S. japonicus* (left), *S. australasicus* (middle) and *S. scombrus* (right), obtained by gel filtration, were chromatographed. The peaks containing parvalbumin are indicated by arrows.

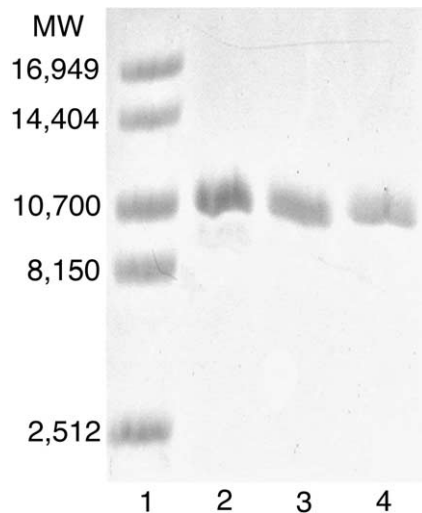


Fig. 2. SDS-PAGE of the purified preparations from three species of mackerels. Lane: 1, standard proteins; 2, preparation from *S. japonicus*; 3, preparation from *S. australasicus*; 4, preparation from *S. scombrus*.

al., 1999; Swoboda et al., 2002). Therefore, the parvalbumins from *S. japonicus*, *S. australasicus* and *S. scombrus* are hereinafter called Sco j 1, Sco a 1 and Sco s 1, respectively, according to the International Nomenclature of Allergens (King et al., 1994).

It should be noted that one serum from patient 5, like two control sera, exhibited no IgE reactivities to any of Sco j 1, Sco a 1 and Sco s 1 (Fig. 4). In our preliminary ELISA experiments using collagen and parvalbumin purified from the white muscle of bigeye tuna *Thunnus obesus*, the patient 5 serum was found to react to collagen but not to parvalbumin. This result supports our previous finding that some Japanese fish-allergic patients recognize not parvalbumin but collagen (Hamada et al., 2001).

Although significant differences in IgE reactivity were observed among the four sera from patients 1–4, Sco j 1, Sco a 1 and Sco s 1 were judged to have almost the same potency in IgE binding ability from their reactivities with each patient serum (Fig. 4). This suggests that three species of mackerels are almost equally allergenic to patients recognizing parvalbumin as an allergen if there is no significant difference in parvalbumin content among the mackerels. Since mackerels are generally considered to be most frequently involved in incidents of IgE-mediated fish allergy in Japan, it is important to clarify whether mackerel parvalbumins are more allergenic than those from other groups of fish. It is also worth mentioning that mackerels are often responsible for allergy-like food poisoning, in which non-immunological adverse reactions are induced by histamine produced through bacterial decarboxylation of free histidine. This may mislead to a concept that mackerels are especially allergenic fish. Therefore, a future questionnaire on fish allergy, strictly diagnosed to be mediated by IgE, is also desired.

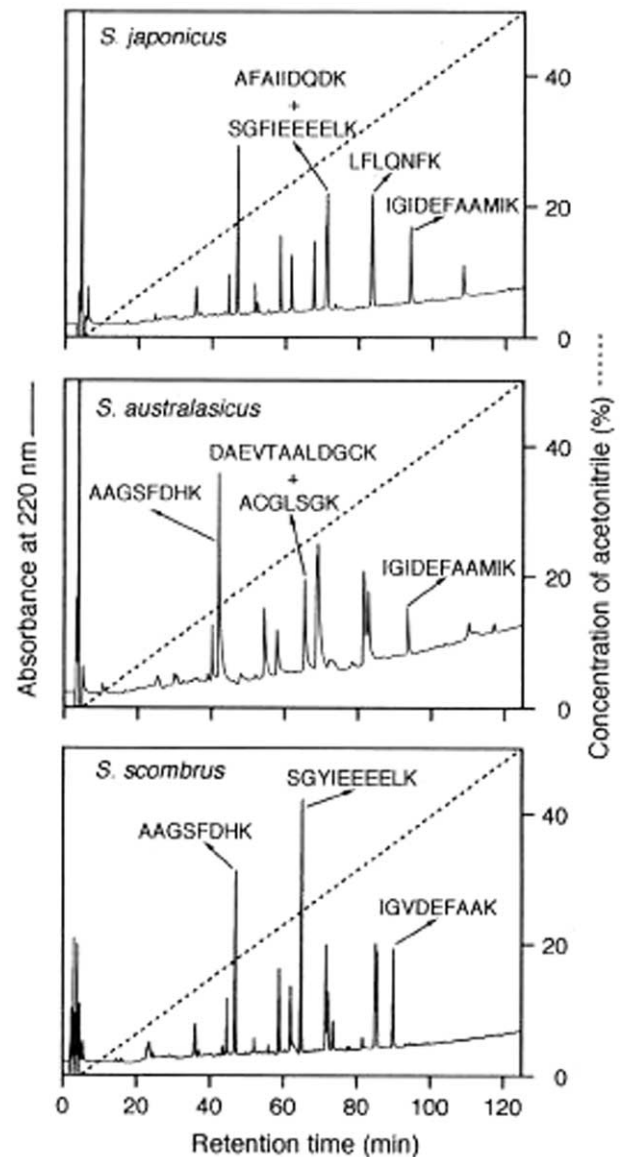


Fig. 3. Separation of lysylendopeptidase fragments from the purified preparations of *S. japonicus* (upper), *S. australasicus* (middle) and *S. scombrus* (lower) by reverse-phase HPLC on TSKgel ODS-120T. The determined amino acid sequences of some peptide fragments are included.

ELISA inhibition experiments were performed using three sera from patients 1, 2 and 4. As shown in Fig. 5, preadsorption of patient sera with Sco j 1, Sco a 1 or Sco s 1 remarkably reduced their IgE reactivity to Sco j 1, demonstrating the antigenic cross-reactivity among the mackerel parvalbumins. The observed cross-reactivity suggests that the mackerel parvalbumins share the same or closely similar IgE-binding epitopes. Cross-reactivity among various species of fish has so far been well established and parvalbumin has been assumed to be a cross-reactive allergen (Aas, 1966; de Martino et al., 1990; Bernhisel-Broadbent et al., 1992a,b; Pascual et al., 1992; Hansen, et al., 1997; Swoboda et al., 2002). In previous

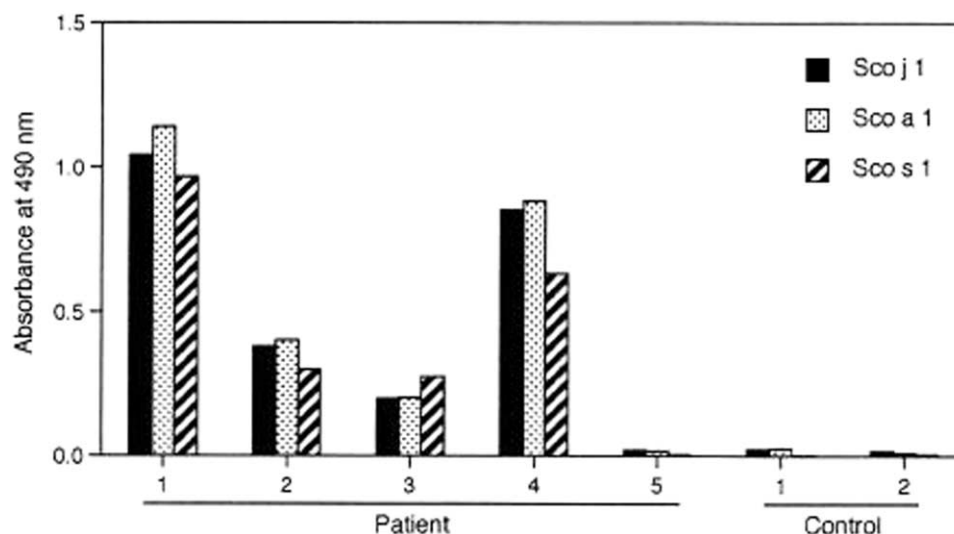


Fig. 4. Analysis by ELISA of the IgE reactivity of patient and control sera to mackerel parvalbumins. Each parvalbumin was coated on the plate at a concentration of 0.1 μ g/well and reacted with sera (1:50 dilution).

studies, however, cross-reactivity has been mostly examined either between crude extracts from fish or between a certain purified parvalbumin and crude extracts. In order to understand the cross-reactivity among fish at the molecular level, future study is needed to clarify the cross-reactivity among purified parvalbumin preparations from various species of fish, as performed with the mackerel parvalbumins in this study.

3.3. Cloning of *S. japonicus* parvalbumin cDNA and deduced amino acid sequence

A cDNA library was constructed from the white muscle of *S. japonicus* and screened with the anti-frog muscle parvalbumin monoclonal antibody. A total of 20 positive clones were obtained and nucleotide sequencing of nine clones revealed that all encoded the same parvalbumin, which is assignable to Sco j 1. The nucleotide sequence (deposited in the DDBJ/EMBL/GenBank nucleotide sequence database with the accession number AB091470) of the longest clone including the entire coding region, together with the deduced amino acid sequence, is shown in Fig. 6. The cDNA comprises 38 bp of the upstream, 327 bp of the coding region and 276 bp of the 3'-untranslated region.

Some fish are known to contain several isoforms of parvalbumin. For example, two distinct isoforms of parvalbumin exist in Atlantic salmon as evidenced by cDNA cloning experiments (Lindström et al., 1996). As for carp, two isoforms found in cDNA cloning experiments (Swoboda et al., 2002) are different from two isoforms previously purified and sequenced (Kretsinger, 1972; Coffee and Bradshaw, 1973), suggesting the presence of at least four isoforms. In this study, however, only one parvalbumin component (Sco j 1) was detected in *S. japonicus* during both chromatographic purification

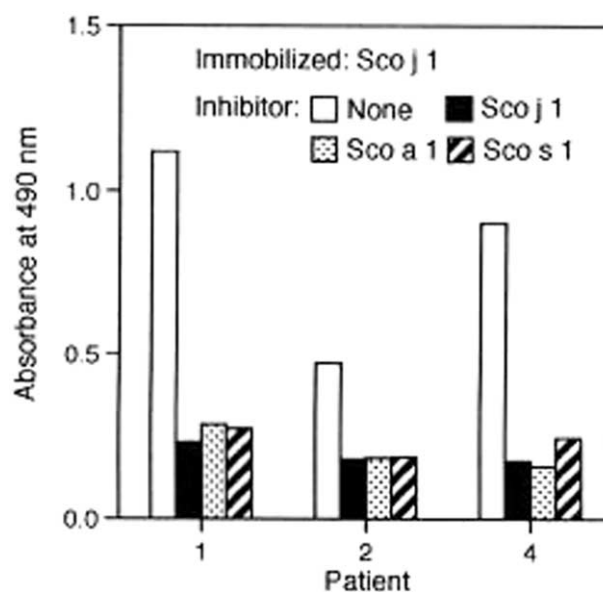
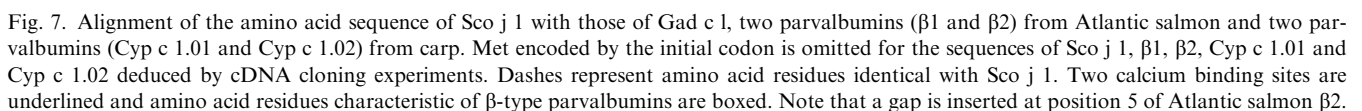


Fig. 5. Inhibition of the IgE reactivity of patient sera to Sco j 1 by Sco j 1, Sco a 1 and Sco s 1. The plate was coated with Sco j 1 (0.1 μ g/well). Prior to addition to the plate, each serum (1:25 dilution) was incubated with an equal volume of Sco j 1, Sco a 1 or Sco s 1 solution (10 μ g/ml).

steps and cDNA cloning experiments. It is thus assumed that isoforms other than Sco j 1 are absent in *S. japonicus* or trace if present. This appears to be true for *S. australasicus* and *S. scombrus*, since only Sco a 1 and Sco s 1 were purified from the former and the latter, respectively.

As aligned in Fig. 7, the deduced amino acid sequence (composed of 108 residues) of Sco j 1 shows high identities (58–76%) with those of parvalbumins from cod (Elsayed and Bennich, 1975), β 1 and β 2 from Atlantic salmon (Lindström et al., 1996) and Cyp c 1.01 and Cyp c 1.02 from carp (Swoboda et al., 2002), which have already been identified as the major allergens in the

Fig. 6. Nucleotide sequence of Sco j 1. Deduced amino acid sequence is denoted below the nucleotide sequence. Both initiation and stop codons are underlined.



IgE-binding epitopes of allergens are deeply associated with the manifestation of allergic reactions and hence their elucidation is particularly important for the development of future immunotherapy of allergies and also for the development of hypoallergenic foods in the case of food allergens. However, studies on IgE-binding epitopes of fish parvalbumins have so far been con-

ducted only with Gad c 1. Based on the IgE-binding abilities of enzymatic peptide fragments and some synthetic peptides, at least five segments (13–32, 33–44, 41–64, 65–74 and 88–96) are assumed to contain IgE-binding epitopes (Elsayed and Apold, 1983). The amino acid sequences of these five segments of Gad c 1 are deserving of comparison with those of the other five parvalbumins shown in Fig. 7. In the segment 88–96, Sco j 1, Cyp c 1.01 and Cyp c 1.02 are completely identical with Gad c 1 and Atlantic salmon β 1 and β 2 have only two or three alterations compared to Gad c 1. High sequence similarities (more than 60%) are also observed in the segment 41–64 between Gad c 1 and the other parvalbumins. It should be noted that as for the segment 41–64 of Gad c 1, at least two of the three identical or similar repeated tetrapeptides ($^{41}\text{DELK}^{44}$, $^{51}\text{DEDK}^{54}$ and $^{61}\text{DELK}^{64}$) interspaced by six amino acid residues are indispensable for the binding with IgE, while the intermediate hexapeptides (45–50 and 55–60) are not.

As compared to Gad c 1, all the other parvalbumins have the same sequence or have only one replacement by a homologous residue (for example, in the case of Sco j 1, Leu-43, Glu-52 and Asp-61 of Gad c 1 are replaced by Val-43, Gln-52 and Glu-61, respectively) in each tetrapeptide segment, except that Atlantic salmon β 1 and Cyp c 1.01 have two alterations in the segments 51–54 and 41–44, respectively. In contrast, sequence similarities between Gad c 1 and the other parvalbumins are fairly low (less than 50%) in the other three segments. Perhaps not all of the five IgE-binding epitopes identified for Gad c 1 (Elsayed and Apold, 1983) are of equivalent importance based upon the structural differences observed for several of the segments in other fish parvalbumins. We thus consider that IgE-binding epitopes of fish parvalbumins should be examined with each parvalbumin.

In conclusion, this study demonstrates that parvalbumin is the major and cross-reactive allergen in common with three species of mackerels which are considered to be especially allergenic in Japan. Furthermore, the cDNA encoding the *S. japonicus* parvalbumin (Sco j 1), which would be a useful tool for diagnosis and therapy of fish allergy, was successfully cloned. Based on the deduced amino acid sequence, elucidation of IgE-binding epitopes of Sco j 1 is now under progress using synthetic overlapping peptides covering the entire sequence.

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