JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

# Major Allergen and Its IgE Cross-Reactivity among Salmonid Fish Roe Allergy

YUTAKA SHIMIZU,[†] ATSUSHI NAKAMURA,[†] HIDEKI KISHIMURA,[†] AKIHIKO HARA,[†] KAZUHIKO WATANABE,[‡] AND HIROKI SAEKI*,[†]

Faculty of Fisheries Sciences, Hokkaido University, Hakodate Minatomachi 3-1-1, Hokkaido 041-8611, Japan, and Watanabe Kazuhiko Pediatric Clinic, Hondouri 1-1-13, Shiroishi, Sapporo, Hokkaido 003-0026, Japan

Yolk protein extracts were prepared from four kinds of salmonid fish roes, and the proteins that reacted with IgE were screened by immunoblotting using sera from 20 patients allergic to chum salmon roe. IgE cross-reactivities among the salmonid yolk proteins were also investigated by competitive ELISA. The results were as follows: (1) The major protein components in salmonid roes were lipovitellin and $\beta'$-component, which are subfragments of vitellogenin. (2) Most sera from the patients showed IgE reactivity to $\beta'$-component in all yolk protein extracts, and some of them also reacted to lipovitellin heavy chain or its light chain. (3) Salmonid $\beta'$-component showed high similarity (>90%) in the N-terminal amino acid sequence. (4) All of the salmonid yolk protein extracts inhibited the IgE reaction between patient sera and the chum salmon $\beta'$-component. These findings indicate that the $\beta'$-component in salmonid roe is a common major allergen with strong IgE cross-reactivity.

**KEYWORDS: Food allergen; *β′*-component; cross-reactivity; lipovitellin; salmon roe allergy**

## INTRODUCTION

The increase in the number of food allergy cases is a serious medical problem in Japan. According to a recent survey (2004−2005), 10.4% of elementary school children and their families ($n = 34{,}441$) have some kind of food allergy. Seafood is recognized as a major allergen (*1*), a trend closely related to seafood consumption in Japan, where more than 500 kinds of marine bioresources are consumed, and new kinds of hypersensitivities to seafood have been reported in the past decade. Salted fish roes, including those of salmon, walleye pollock, and herring, are commonly consumed in Japan, and many cases of salmon roe allergy, particularly among children, have been reported in the past decade (*1, 2*). Therefore, salmon roe has been listed as a food that is recommended to be labeled for potential allergic reactions in an amendment of the 2002 Japanese food sanitation law (*3*).

There have been reports of individuals experiencing immediate allergic reactions to the consumption of king salmon caviar (*4*), Russian beluga caviar (*5*), and the roe of white fish and rainbow trout (*6*). IgE cross-reactivities among fish roes, such as those from salmon, herring, and walleye pollock, have been reported in case studies (*7*). Additionally, preliminary data from immunoblotting and ELISA obtained in this research suggest that a vitellogenin fragment, $\beta'$-c, is a major allergen in chum salmon roe (*8*). However, compared to the ample information available regarding fish and shellfish allergens, there is little scientific information about fish roe allergens.

The major allergens of hen eggs are egg-white proteins, such as ovalbumin, ovomucoid, and lysozyme (*9*−*11*). However, fish roe contains no part that is equivalent to egg white, the yolk being the major component. Teleost fish roe contains three major yolk proteins: lipovitellin, phosvitin, and $\beta'$-c. Vitellogenin, a precursor of these yolk proteins, is synthesized in the fish liver, carried in the bloodstream, accumulated in oocytes, and fragmented with oocyte growth (*12*−*15*). In this work, we clearly identified the major and minor allergens in four kinds of salmonid fish roes and investigated the IgE cross-reactivity of the allergens among salmonid fish roes by competitive ELISA using sera from patients.

## MATERIALS AND METHODS

**Fish Roes.** Fish roes were obtained from fresh chum salmon (*Oncorhynchus keta*), rainbow trout (*Oncorhynchus mykiss*), Sakhaline taimen (*Hucho perryi*), and Japanese charr (*Salvelinus leucomaenis leucomaenis*). The salmonid roes were washed with 0.16 M of cold NaCl and frozen at −60 °C until use.

**Patient Sera.** Sera from 20 patients diagnosed with a salmon roe allergy were selected for this study (age range, 2−11 years; male, 10; female, 10). Sera from nonallergic individuals (age range, 22−43; male, 4) were used as the control. The clinical data of the patients and the nonallergic individuals are listed in **Table 1**. Each serum was subjected to CAP-RAST (ImmunoCAP, Phadia AB, Uppsala, Sweden) to determine the total IgE level and specific IgE level for chum salmon roe allergy. In the diagnostic system, the whole extract of chum salmon roe was used as a solid-phase antigen (Phadia K.K., Tokyo, Japan).

* Corresponding author. Tel: 81-138-40-5516. Fax: 81-138-40-5516. E-mail: saeki@fish.hokudai.ac.jp.
[†] Hokkaido University.
[‡] Watanabe Kazuhiko Pediatric Clinic.

10.1021/jf8031759 CCC: $40.75 
Published on Web 02/18/2009

**Table 1.** List of Allergic Patients Hypersensitive to Chum Salmon Roe[a]

| serum | age | sex | CAP-RAST (Class) | total IgE (IU/mL) | hypersensitivity reaction |
|---|---|---|---|---|---|
| P1 | 4 | F | * | 2677 | Ur |
| P2 | 6 | F | 2 | 1012 | Ur |
| P3 | 5 | F | 4 | 593 | OAS |
| P4 | 5 | F | 6 | * | Ur, Vom, AD, BA |
| P5 | 2 | F | 4 | 1129 | Ur |
| P6 | 11 | F | 6 | 3452 | AD |
| P7 | 1 | F | 4 | 424 | AD |
| P8 | 1 | M | 4 | 66 | AD |
| P9 | 5 | F | 6 | 911 | Ur |
| P10 | 2 | M | 5 | 2398 | AD, BA |
| P11 | 6 | M | 4 | 1017 | BA, OAS |
| P12 | 1 | M | 4 | 221 | AD |
| P13 | 6 | F | 6 | 822 | OAS |
| P14 | 2 | F | 5 | 349 | AD |
| P15 | 1 | M | 5 | 524 | AD |
| P16 | 3 | M | 5 | * | QE |
| P17 | 2 | M | 5 | 7030 | AD |
| P18 | 2 | M | 4 | 9120 | AD |
| P19 | 5 | M | 4 | 5210 | BA, AD |
| P20 | 3 | M | 4 | 2131 | BA, AD |

[a] AD, atopic dermatitis; BA, bronchial asthma; OAS, oral allergy syndrome; QE, Quincke's edema; Ur, urticaria; Vom, vomit; *, no data.

The patient sera were frozen at < −60 °C for 2 to 12 months and were then thawed and mixed with the same volume of PBS containing 0.2% $NaN_3$. They were stored at 4 °C until use.

**Antibody against Fish Yolk Proteins.** The purified chum salmon $\beta'$-c and lipovitellin were emulsified with Freund's complete adjuvant (Pierce, Rockford, IL). The emulsions were injected into rabbits (NZW, male, 3 months old) once a week for four weeks. One week after the fourth injection, rabbit blood was gathered and centrifuged at 3,000*g* for 15 min to collect the supernatant. Forty percent of saturated ammonium sulfate at the final concentration was added to the supernatant, and the mixture was centrifuged at 30,000*g* for 30 min. The supernatant was dialyzed (MWCO, 12k−14k) into PBS, and the same volume of PBS containing 0.2% $NaN_3$ was added. Antibodies against fish yolk proteins thus obtained (a-$\beta$, a-Lv) were stored at 5 °C until use. The animal experiment was performed according to the Guidelines Concerning Animal Experiments at Hokkaido University.

**Preparation of Fish Roe Proteins.** The thawed roes were homogenized in 5-fold weight of 0.5 M NaCl and 20 mM Tris-HCl (pH 8.0) using a Potter homogenizer. The homogenates were centrifuged at 2,000*g* for 15 min to remove the floating oil layer and further centrifuged at 20,000*g* for 30 min. The supernatants as YPE were frozen at −30 °C until use.

$\beta'$-c was prepared from chum salmon roe according to a modified method of Hara et al. (*16, 17*). Briefly, YPE was dropped into 10 volumes of cold distilled water. The precipitate generated in this step was collected by centrifugation at 15,000*g* for 30 min and dissolved in 0.5 M NaCl and 20 mM Tris-HCl (pH 8.0). Sixty-seven percent of saturated ammonium sulfate at the final concentration was added to the salt-soluble fraction and centrifuged at 30,000*g* for 30 min. The precipitate was redissolved in 0.5 M NaCl and 20 mM Tris-HCl (pH 8.0) and loaded onto a Sephacryl S-200HR column (60 × Ø 1.6 cm, GE Healthcare, Piscataway, NJ) to purify $\beta'$-c. The protein fractions were detected at 280 nm, and the concentration was determined by the Biuret method (*18*). All steps were performed at temperatures below 5 °C, and the purified proteins were frozen at −30 °C until use.

**SDS−PAGE Analysis.** SDS−PAGE was performed by the method of Laemmli (*19*), using 4.5% and 12.5% acrylamide slab gels for the stacking and resolving gels, respectively. The protein bands were stained with 0.25% Coomassie Brilliant Blue R (Sigma, St. Louis, MO) dissolved in 9% acetic acid and 45% methanol.

**Immunoblotting.** The proteins in salmonid YPE that reacted with a-$\beta$, a-Lv, or patients' sera were detected by immunoblotting. Proteins separated by SDS−PAGE were transferred onto a polyvinylidene difluoride membrane (Immobilon-P, Millipore, Billerica, MA) using a semidry blotting system (ATTO, Tokyo, Japan). The membrane was soaked in a blocking buffer (3% casein in TBS) at room temperature for 1 h. After blocking, the membrane was incubated with the patients' sera (diluted 1:50−200 with the blocking buffer), and the antibodies (a-$\beta$, a-Lv) were diluted (1:30,000) with the blocking buffer at 5 °C overnight. After being washed three times with TTBS and TBS, the IgE or IgG binding to the protein was reacted with the peroxidase-conjugated rabbit antihuman IgE antibody (Dako, Amsterdam, Denmark) or peroxidase-conjugated goat antirabbit IgG antibody (BioRad, Hercules, CA) at 37 °C for 3 h. After being washed three times with TTBS and TBS, the reacted blots were detected with an ECL photosystem (GE Healthcare, Piscataway, NJ) as a detection reagent.

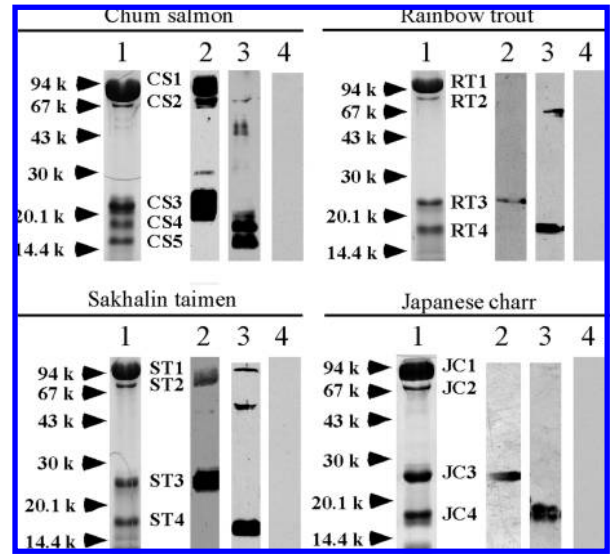


**Figure 1.** SDS−PAGE patterns of salmonid roe proteins and their Western blotting using rabbit antisera against chum salmon lipovitellin (a-Lv), $\beta'$-c (a-$\beta$). Salmonid roe extracts (lane 1) were reacted with a-Lv (lane 2) and a-$\beta$ (lane 3) in Western blotting. Serum of rabbits without immunization (lane 4) was used as a control. Several protein bands of each salmonid roe were tagged as CS1-CS5 (chum salmon), RT1-RT4 (rainbow trout), ST1-ST4 (Sakhalin taimen), and JC1-JC4 (Japanese charr).

**Competitive ELISA.** Cross-reactivity among salmonid YPEs was investigated by competitive ELISA using patients' sera. A 96-well ELISA plate (IWAKI, Tokyo, Japan) was coated with 2.5 $\mu$g/mL $\beta'$-c (100 $\mu$L/well) that had been dissolved in PBS and incubated overnight at 5 °C. After being washed with TPBS, the residual blocking sites in each well were coated with a blocking buffer (1% casein in PBS) at 37 °C for 2 h. Simultaneously, 125 $\mu$L of patients' sera or nonallergic individuals' sera (diluted 1: 25−100 with the blocking buffer) was mixed with equal volumes of the YPE as an inhibitor (0.002−200 $\mu$g/mL diluted with the blocking buffer). After being incubated at 37 °C for 2 h, 70 $\mu$L of each solution was placed into the $\beta'$-c-coated ELISA plate and incubated at 37 °C for 2 h. After the plate was washed with TPBS, 100 $\mu$L/well of peroxidase-conjugated rabbit antihuman IgE antibody diluted with the blocking buffer (1:5000) was added to each well and incubated at 37 °C for 1.5 h. The enzyme−substrate reaction was performed using 0.04% *o*-phenylendiamine dihydrochloride and 0.05% $H_2O_2$ in a 50 mM phosphate−citrate buffer (pH 5.0) at 25 °C for 20 min. The reaction was terminated by adding 100 $\mu$L/well of 4 N sulfuric acid. The detection of the enzyme reaction was carried out by measuring absorbance at 492 nm using a microplate reader (MTP-300, Corona Electric, Ibaraki, Japan). The loss of the specific IgE-binding ability of the patients' sera resulting from the treatment with the inhibitors was represented by calculating the inhibition rate using the following formula: inhibition rate (%) = $(X - Y) \div (X - Z) \times 100$, where $X$ is the absorbance of each patient's serum without the inhibitors, and $Y$ and $Z$ are the absorbance of the patients' sera and that of nonallergic individuals' sera treated with various concentrations of inhibitors, respectively.

**Statistical Analysis.** The results of each measurement in **Figure 3** were the average of three determinations, and error bars corresponded

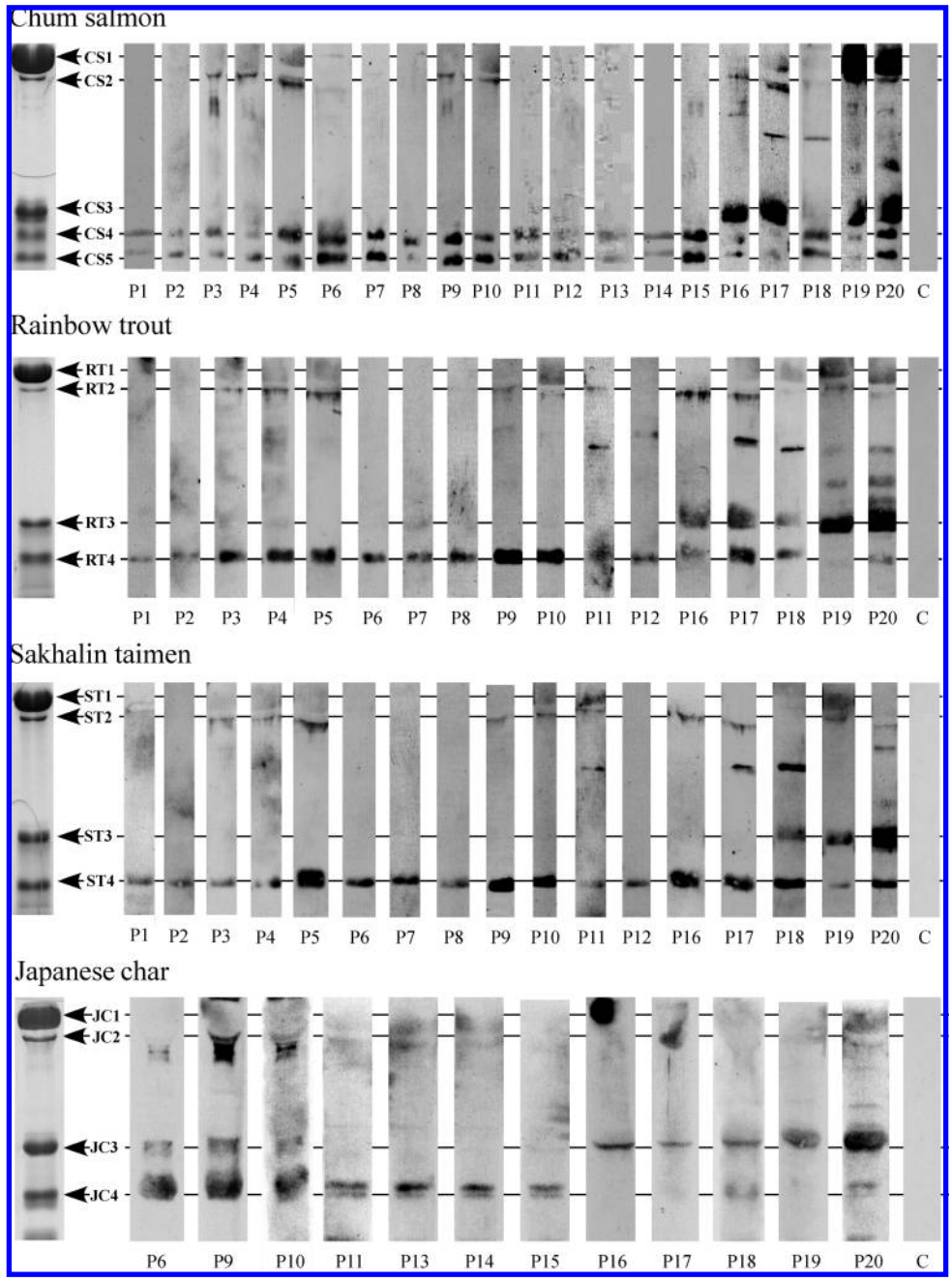


**Figure 2.** Specific IgE reactivity between salmonid yolk proteins (chum salmon, rainbow trout, Sakhalin taimen, and Japanese charr) and sera of patients allergic to fish roe in immunoblotting. Chum salmon roe extract was reacted with sera of P1−P20, rainbow trout roe, and Sakhalin taimen roe extracts were reacted with sera of P1−P12 and P16−P20, and a Japanese charr roe extract was reacted with sera of P6, P9−P11, and P13−P20. Sera from nonallergic individuals were also examined as a negative control (lane C).

to the standard deviations. Statistical differences were tested using Dunn's procedure as a multiple comparison procedure (Bonferroni/Dunn method) at the 5% significance level with the Statcel software ver. 1.0 (OMS-Publishing, Saitama, Japan).

**N-Terminal Amino Acid Sequence.** The protein-blotting membranes were stained with 0.1% Coomassie Brilliant Blue R in 30% methanol and 7.5% acetic acid, and the stained protein bands were subjected to the automatic Edman sequence analyzer (Procise 492, Perkin-Elmer, Waltham, MA).

## RESULTS

**SDS−PAGE Analysis and IgG-Immunoblotting of Salmonid Roe Proteins. Figure 1** shows the SDS−PAGE patterns of the YPE of four salmonid fish roes. In lane 1, five major components (CS1-CS5) were observed in chum salmon, and four major components (RT1-RT4, ST1-ST4, and JC1-JC4) were observed in three other salmonid species: rainbow trout, Sakhalin taimen, and Japanese charr. Since high molecular bands, CS1, CS2, and ST1, were reacted with a-Lv in lane 2, they were estimated as the lipovitellin heavy chain (CS1 and ST1) and its subfragments (CS2). Components of 20−25 kDa (CS3, RT3, ST3, and JC3) that reacted with a-Lv in lane 2 were identified as the lipovitellin light chain. Furthermore, 15−20-kDa-protein bands (CS4, CS5, RT4, ST4, and JC4) were identified as $\beta'$-c by the reaction with a-$\beta$ (lane 3). As confirmed in lane 4, no protein component reacted with the serum of rabbit without immunization. These results indicate that lipovitellin and $\beta'$-c are the major components in YPE.

**Table 2.** N-Terminal 20 Amino Acids Sequence of Salmonid Fish $\beta'$-Component[a]

| species | N-terminal sequence | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| chum salmon (CS4) | E | V | N | A | V | K | C | S | M | V | G | D | T | L | T | T | F | N | N | R |
| chum salmon (CS5) | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| rainbow trout (RT4) | • | • | • | • | • | • | • | • | • | • | R | • | • | • | • | • | • | • | • | K |
| Sakhalin taimen (ST4) | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | R |
| Japanese charr (JC4) | • | • | • | • | • | Q | • | • | • | • | D | • | • | • | • | • | • | • | • | C |

[a] The abbreviations (CS4, CS5, RT4, ST4, and JC4) are shown in **Figure 1**.

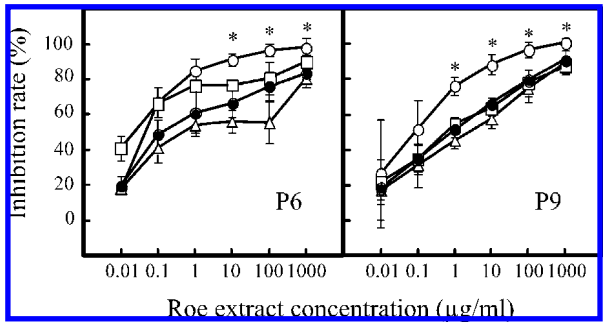


**Figure 3.** Inhibitory effect of salmonid roe extracts on the reaction between specific IgE and $\beta'$-c. Patients' sera were mixed with YPE of salmonid roes and reacted with chum salmon $\beta'$-c in competitive ELISA. (○), chum salmon; (△), rainbow trout; (□), Sakhalin taimen; (●), Japanese charr. Data are expressed as the mean ± standard deviation ($n = 3$). Asterisks indicate that the data of chum salmon YPE are significantly different from those of other YPEs ($p < 0.05$).

As shown in **Table 2**, the N-terminal amino acid sequence of CS4 and CS5 was EVNAVKCSMVGDTLTTFNNR and showed high similarity (>90%) to the $\beta'$-c of other salmonid fish roes. CS5 (16 kDa) seemed to be a subfragment of CS4 (18 kDa) because their N-terminal sequences were identical.

**IgE-Immunoblotting Analysis of Salmonid Yolk Proteins.** Specific IgE reactivity to salmonid YPE was investigated by immunoblotting using sera from 20 patients (**Figure 2**). As clearly shown in the figure, the sera of nonallergic individuals (C) showed no IgE reactivity to all salmonid YPEs; the same result was observed in the other three control sera (data not shown). However, the allergic patients' sera showed specific IgE reactivity to YPE. In chum salmon, all patients' sera reacted to $\beta'$-c, and some sera reacted to both lipovitellin and $\beta'$-c. That is, IgE reactivity to CS4 and CS5 was observed in all patients' sera. In addition, some of the sera contained specific IgE that reacted with CS1 (P5, P10, P17, P19, and P20), CS2 (P3, P4, P5, P9, P10, P16, P17, P19, and P20), and CS3 (P16, P17, P19, and P20). In rainbow trout, specific IgE reactivity to RT4 was observed in the sera of 16 allergic patients, with the exception of P19. Sera from five patients (P16, P17, P18, P19, and P20) showed IgE reactivity to RT3, and sera from 10 patients showed IgE reactivity to RT1 or RT2. In Sakhalin taimen, the sera from all allergic patients ($n = 17$) showed IgE reactivity to ST4, and sera from three patients (P18, P19, and P20) reacted with ST3. Furthermore, IgE reactivity to ST1 or ST2 was observed in the sera of 10 patients. In Japanese charr, the sera from six patients ($n = 12$, P9, P10, P11, P13, P14, and P20) apparently showed IgE reactivity to JC1 or JC2. Additionally, the sera from nine allergic patients contained the specific IgE that reacted to JC4, and sera from eight allergic patients reacted to JC3.

The IgE reactivity of each patient's serum to four salmonid YPEs is summarized in **Table 3**. The patients showed two types of salmon roe allergies, one with IgE reactivity to $\beta'$-c, and the other with IgE reactivity to lipovitellin. Forty-five percent (9/20) of the patients' sera reacted to lipovitellin (CS1, CS2, and CS3) of chum salmon YPE and to other salmonid lipovitellin. However, all of the patients' sera contained a specific IgE that reacted with $\beta'$-c from chum salmon, and each serum also had IgE reactivity to other salmonid $\beta'$-cs (RT4, ST4, and JC4). In addition, patients' sera that reacted with lipovitellin (P16, P17, P18, P19, and P20) showed IgE reactivity to $\beta'$-c. These results clearly indicate that $\beta'$-c is a common major allergen in salmonid fish roes.

**IgE Cross-Reactivity among Salmonid $\beta'$-c.** The inhibitory effect of salmonid YPE on the reaction between the patients' sera (P6 and P9) and chum salmon $\beta'$-c was examined to clarify IgE cross-reactivity among the major salmonid allergens. **Figure 3** shows the results of competitive ELISA using salmonid YPEs as inhibitor antigens. When chum salmon YPE was mixed with the serum of P6, the IgE reaction between the patient serum and $\beta'$-c was effectively inhibited, with a rise in the YPE concentration. The inhibition rate reached the maximum (>95%) at 100 $\mu$g/mL of the chum salmon YPE. The inhibitory effect with the concentration dependence was also observed in other salmonid YPEs (rainbow trout, Sakhalin taimen, and Japanese charr), and the inhibition rate at 100 $\mu$g/mL of all YPEs reached >70%. Apparently, the inhibition was caused only by $\beta'$-c in YPE because the serum of P6 contained no lipovitellin-specific-IgE as listed in **Table 3**. Furthermore, the results of competitive ELISA were confirmed in the sera of P9 and four other patients (P4, P10, P17, and P18: data not shown). These results clearly indicate that a strong IgE cross-reactivity exists among salmonid $\beta'$-cs.

## DISCUSSION

The sera of patients allergic to chum salmon roe showed IgE reactivity to both lipovitellin and $\beta'$-c, major components in the YPE of salmonid fish roes (**Figure 1**). In the case of chum salmon YPE, all of the patients' sera showed specific IgE reactivity to both $\beta'$-c subunits (16 kDa and 18 kDa), and 45% of the sera showed specific IgE reactivity to lipovitellin. These results suggest that $\beta'$-c and lipovitellin cause food allergies and that $\beta'$-c is a major allergen in chum salmon roe. Although only chum salmon $\beta'$-c consisted of two subunits in SDS−PAGE analysis (**Figure 1**), there was no difference in the IgE reactivity between the two $\beta'$-c subfragments in immunoblotting (**Figure 2**). The 16 kDa $\beta'$-c band seems to be a subfragment of the 18 kDa component lacking a C-terminal region because their N-terminal sequences coincide, as shown in **Table 2**.

All of the patient sera that reacted with chum salmon $\beta'$-c also showed IgE reactivity to other salmonid $\beta'$-c's, as summarized in **Table 3**. This result suggests that $\beta'$-c is a common allergen in salmonid fish roe-induced hypersensitivity. In addition, the cross-reactivity of $\beta'$-c between chum salmon and other salmonid fish roes was confirmed, as shown in **Figure 3**. This finding corresponds with the fact that sera from patients experiencing chum salmon roe allergies tend to have IgE reactivity to all the salmonid $\beta'$-c's. Interestingly, the inhibitory effects of rainbow trout, Sakhalin taimen, and Japanese charr

**Table 3.** Salmonid Fish Yolk Proteins Reacted with Sera of Allergic Patients[a]

| | chum salmon | | | | | rainbow trout | | | | Sakhalin taimen | | | | Japanese charr | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| serum | CS1 | CS2 | CS3 | CS4 | CS5 | RT1 | RT2 | RT3 | RT4 | ST1 | ST2 | ST3 | ST4 | JC1 | JC2 | JC3 | JC4 |
| P1 | − | − | − | ++ | ++ | − | − | − | + | − | − | − | + | * | * | * | * |
| P2 | − | − | − | ++ | ++ | − | − | − | + | − | − | − | + | * | * | * | * |
| P3 | − | + | − | ++ | ++ | + | + | − | ++ | − | + | − | ++ | * | * | * | * |
| P4 | − | + | − | ++ | ++ | + | + | − | ++ | − | + | − | ++ | * | * | * | * |
| P5 | + | + | − | ++ | ++ | + | + | − | ++ | − | + | − | ++ | * | * | * | * |
| P6 | − | − | − | ++ | ++ | − | − | − | ++ | − | − | − | ++ | − | − | + | ++ |
| P7 | − | − | − | ++ | ++ | − | − | − | ++ | − | − | − | ++ | * | * | * | * |
| P8 | − | − | − | + | + | − | − | − | ++ | − | − | − | ++ | * | * | * | * |
| P9 | − | + | − | ++ | ++ | − | + | − | ++ | − | + | − | ++ | − | + | + | ++ |
| P10 | + | + | − | + | + | + | + | − | ++ | + | + | − | ++ | − | + | + | ++ |
| P11 | − | − | − | + | + | − | + | − | + | + | + | − | + | + | + | − | ++ |
| P12 | − | − | − | ++ | ++ | − | − | − | ++ | − | − | − | ++ | * | * | * | * |
| P13 | − | − | − | ++ | ++ | * | * | * | * | * | * | * | * | + | + | − | ++ |
| P14 | − | − | − | ++ | ++ | * | * | * | * | * | * | * | * | + | + | − | ++ |
| P15 | − | − | − | ++ | ++ | * | * | * | * | * | * | * | * | − | − | − | ++ |
| P16 | − | + | + | + | + | − | + | + | + | − | + | − | ++ | − | − | + | − |
| P17 | + | + | ++ | + | + | − | + | ++ | ++ | − | + | − | ++ | − | − | + | − |
| P18 | − | − | − | ++ | ++ | − | − | + | ++ | − | − | + | ++ | − | − | + | + |
| P19 | ++ | ++ | ++ | + | + | + | + | ++ | − | + | + | ++ | + | − | − | ++ | − |
| P20 | ++ | ++ | ++ | ++ | ++ | + | − | ++ | + | − | + | ++ | ++ | + | + | ++ | + |
| a-Lv | ++ | ++ | ++ | − | − | − | − | + | − | ++ | − | ++ | − | − | − | ++ | − |
| a-b | − | − | − | ++ | ++ | − | − | − | ++ | − | − | − | ++ | − | − | − | ++ |

[a] The abbreviations (CS4, CS5, RT4, ST4 and JC4) are shown in **Figure 1**. ++, strong positive; +, positive; −, negative; *, no data.

YPEs were weak compared with that of chum salmon YPE. The inhibition rates of chum salmon YPE(1−1000 $\mu$g/mL) were significantly higher than those of other salmonid YPEs as described in **Figure 3**. This result suggests that chum salmon $\beta'$-c contains a specific epitope, which does not exist in other salmonid roes (species-specific epitope). It seems that the IgE reaction related to common epitopes in salmonid $\beta'$-c was inhibited by all salmonid YPEs and that the three kinds of salmonid YPEs did not block the species-specific epitope. Although there was high homology in the N-terminal amino acid sequence in salmonid $\beta'$-cs (**Table 2**), the SDS−PAGE pattern of chum salmon $\beta'$-c was different from that of rainbow trout, Sakhalin taimen, and Japanese charr (**Figure 1**). Therefore, each $\beta'$-c must have commonly cross-reacting sequential epitopes and species-specific epitopes reacting to a specific IgE.

In Japan, seafood is much more commonly consumed than livestock is; thus, information on IgE cross-reactivity among foods is important in order to reduce seafood allergies. In general, allergens have several epitopes, and the target of a specific IgE depends on the patient. However, as shown in **Table 3**, the sera of allergic patients with hypersensitivity to chum salmon roe showed IgE reactivity to other salmonid $\beta'$-c's. This finding indicates that the sera of the allergic patients contained a specific IgE, which reacted with the common epitopes in salmonid $\beta'$-c's. Identifying the common and species-specific epitopes obtained by immunoblotting and competitive ELISA would assist in understanding IgE cross-reactivities among other kinds of fish roe allergies. Kondo et al. (*7*) reported that some patients allergic to salmon roe were also hypersensitive to the roe of pollock and herring. If $\beta'$-c is the major allergen in various kinds of fish roe and the common and species-specific epitopes are identified, it will be possible to predict the IgE cross-reactivity in each patient. Such information will be useful for planning a dietary cure and a therapeutic strategy. Furthermore, Kiljunen et al. (*6*) and Kondo et al. (*7*) have reported no relationship between fish muscle consumption and fish roe allergies. This result is further supported by the fact that vitellogenin, synthesized in the liver and carried in the bloodstream (*12*−*15*), does not exist in fish muscle. However, vitellogenin may contaminate muscle tissue in female salmon because the blood of mature salmon contains a high concentration of vitellogenin (*15*). Therefore, patients allergic to salmon roe should be cautious of any intake of mature female salmonid fish during the spawning season.

## ABBREVIATIONS USED

a-$\beta$, antichum salmon $\beta'$-c rabbit IgG; a-Lv, antichum salmon lipovitellin rabbit IgG; $\beta'$-c, $\beta'$-component; CAP-RAST, capsulated hydrophilic carrier polymer−radioallergosorbent test, ELISA, enzyme-linked immunosorbent assay; PBS, phosphate buffered saline (pH 7.5); SDS−PAGE, sodium dodecylsulfate−polyacrylamide gel electrophoresis; TBS, 20 mM Tris-HCl (pH 7.5) containing 150 mM NaCl; TTBS, TBS containing 0.05% Tween 20; TPBS, PBS containing 0.05% Tween 20; YPE, yolk protein extract.

## ACKNOWLEDGMENT

We thank Dr. Etsuro Yamaha (Nanae Fresh-Water Laboratory Field Science Center for Northern Biosphere, Hokkaido University) for supplying the sample fish roes and Dr. Naoshi Hiramatsu (Faculty of Fisheries Sciences, Hokkaido University) for his suggestion concerning fish roe protein. The amino acid sequences were analyzed by Tomohiro Hirose and Akira Miyao (Center for Instrumental Analysis, Hokkaido University).

## LITERATURE CITED

(1) Statistical analysis on the field survey of food allergy in Kanagawa, Japan (in Japanese), Kanagawa Prefectural Institutes of Public Health, Japan. http://www.eiken.pref.kanagawa.jp/004_chousa/images/061206_allergyreport.pdf.

(2) The Guideline of Food Allergy Diagnosis 2005 (in Japanese). National Hospital Organization, Japan. http://www.hosp.go.jp/resources/content/4772/manual.pdf.

(3) The Foods Labeling Amendment. Ministry of Health, Labour and Welfare, Japan. http://www.mhlw.go.jp/english/topics/qa/allergies/al2.html.

(4) Flais, J. M.; Kim, S. S.; Harris, E. K.; Greenberger, A. P. Salmon caviar-induced anaphylactic shock. *Allergy Asthma Proc.* **2004**, *25*, 233–236.

(5) Untersmayr, E.; Focke, M.; Kinaciyan, T.; Poulsen, K. L.; Boltz-Nitulescu, G.; Scheiner, O.; Jensen-Jarolim, E. Anaphylaxis to Russian beluga caviar. *J. Allergy. Clin. Immunol.* **2002**, *109*, 1034–1035.

(6) Kilijunen, S. M.; Kiistala, R.; Varjonen, E. Severe reaction from roe without concomitant fish allergy. *Ann. Allergy Asthma Immunol*. **2003**, *91*, 413–416.

(7) Kondo, Y.; Kakami, M.; Koyama, H.; Yasuda, T.; Nakajima, Y.; Kawamura, M.; Tokuda, R.; Tsuge, I.; Urisu, A. IgE cross-reactivity between fish roe (salmon, herring and pollock) and chicken egg in patients anaphylactic to salmon roe. *Allergol. Int.* **2005**, *54*, 317–323.

(8) Kubo, T.; Watanabe, K.; Hara, A.; Simizu, H.; Saeki, H. Identification of major allergen in chum salmon roe. *Bull. Fac. Fish Hokkaido Univ.* **2005**, *56*, 55–59.

(9) Hoffman, D. R. Immunochemical identification of the allergens in egg white. *J. Allergy Clin. Immunol*. **1983**, *71*, 481–486.

(10) Bernhisel, B. J.; Dintzis, H. M.; Dintzis, R. Z.; Sampson, H. A. Allergenicity and antigenicity of chicken egg ovomucoid (Gal d III) compared with ovalbumin (Gal d I) in children with egg allergy and in mice. *J. Allergy Clin. Immunol*. **1994**, *93*, 1047–1059.

(11) Hildebrandt, S.; Steinhart, H.; Paschke, A. Comparison of different extraction solutions for the analysis of allergens in hen's egg. *Food Chem*. **2008**, *108*, 1088–1093.

(12) Hiramatsu, N.; Cheek, A. O.; Sullivan, C. V.; Matsubara, T.; Hara, A. Vitellogenesis and Endocrine Disruption. In *Biochemistry and Molecular Biology of Fishes*; Mommsen, T. P., Moon, T. W., Eds.; Elsevier Science Ltd: Amsterdam, The Netherlands, 2005; Vol. 6, pp 431−471.

(13) Hara, A. Iron-binding activity of female-specific serum proteins of rainbow trout (*Salmo gairdneri*) and chum salmon (*Oncorhynchus keta*). *Biochem. Biophys. Acta* **1976**, *427*, 549–557.

(14) Ueda, H.; Hiroi, O.; Hara, A.; Yamauchi, K.; Nagahama, Y. Changes in serum concentrations of steroid hormones, thyroxine, and vitellogenin during spawning migration of the chum salmon *Oncorhynchus keta*. *Gen. Comp. Endocrinol.* **1984**, *53*, 203–211.

(15) Kwon, H. C.; Hara, A.; Mugiya, Y.; Yamada, J. Enzyme linked-immunosorbent assay (ELISA) of vitellogenin in whitespotted charr, Salvelinus leucomaenis. *Bull. Fac. Fish Hokkaido Univ.* **1990**, *40*, 162–180.

(16) Hara, A.; Hirai, H. Comparative studies on immunochemical properties of female-specific serum protein and egg yolk proteins in rainbow trout (*Salmo gairdneri*). *Comp. Biochem. Physiol.* **1978**, *59B*, 339–343.

(17) Hiramatsu, N.; Hara, A. Relationship between vitellogenin and Its related egg yolk proteins in Sakhalin taimen (*Hucho perryi*). *Comp. Biochem. Physiol.* **1996**, *115A*, 243–251.

(18) Gronall, A.; Bardawill, C. J.; David, M. M. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* **1949**, *177*, 751–766.

(19) Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, *227*, 680–685.

**Received for review October 11, 2008. Revised manuscript received January 16, 2009. Accepted January 23, 2009. This work was supported in part by Grant-in-Aid for Scientific Research C (18580200), Ministry of Education, Culture, Sports, Science and Technology, Japan.**

JF8031759