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In vitro digestion of major allergen in salmon roe and its peptide portion with proteolytic resistance

Shingo Fujita [a], Yutaka Shimizu [a], Hideki Kishimura [a], Kazuhiko Watanabe [b], Akihiko Hara [a], Hiroki Saeki [a,*]

[a] Faculty of Fisheries Sciences, Hokkaido University, Minato 3, Hakodate, Hokkaido 041-8611, Japan
[b] Watanabe Kazuhiko Pediatric Clinic, Hondouri 1-1-13, Shiroishi, Sapporo, Hokkaido 003-0026, Japan

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ABSTRACT

A fish yolk protein, β′-component (β′-c), is the major allergen in chum salmon roe. The effect of proteolysis on the allergenicity of β′-c was estimated. Changes in the IgE-binding ability of β′-c upon pepsin and trypsin digestion were investigated by monitoring the proteolytic cleavage. In the pepsin–trypsin digestion of chum salmon yolk protein, the β′-c contained therein was degraded in a manner similar to that of other yolk proteins, but digestion fragments with a molecular mass of >10 kDa remained throughout the digestion process. Specifically, the peptide sequence between 31-Y and 119-Q (10 kDa) was stable to pepsin–trypsin digestion and the portion showed high IgE-binding ability. As a result, pepsin–trypsin digestion had little effect on the IgE-binding ability of β′-c. These results suggest that β′-c reaches the small intestine in the form of high-molecular-mass components with IgE-binding ability *in vivo*.

1. Introduction

Food allergy is a serious medical problem in Japan. The number of people who have hypersensitivity to a specific food is increasing, and the morbidity of seafood-allergic patients has reached 10% among elementary school children and their families (Kanagawa Prefectural Institutes of Public Health, 2006). Food allergy is closely related to dietary habits, and seafood is recognised as a major allergen in Japan, where more than 500 kinds of seafood are consumed. Hypersensitivity to various kinds of fish, shrimp, crab, and processed seafood has been reported, and the number of cases of salmon roe allergy, particularly among children, has increased in the last decade. Therefore, salmon roe has been listed as one of the potential allergen food materials in the Japanese food sanitation law (Ministry of Health, Labour and Welfare, Japan, 2008). Outside of Japan, there have been reports of individuals experiencing immediate allergic reactions to the consumption of king salmon caviar (Flais, Kim, Harris, & Greenberger, 2004), Russian beluga caviar (Untersmayr et al., 2002), and the roe of whitefish and rainbow trout (Kilijunen, Kiistala, & Varjonen, 2003). Immunoglobulin E (IgE) cross-reactivities among fish roes, such as those from salmon, herring, and walleye pollock (Kondo et al., 2005), have been reported in case studies. Therefore, we need to recognise fish roe as a potential allergenic seafood.

Teleost roe contains three major yolk proteins, lipovitellin (Lv), phosvitin (Pv), and β′-component (β′-c) (Hiramatsu & Hara, 1996; Matsubara & Sawano, 1995), which are utilised as sources of embryonic nutrients in oviparous vertebrates (Hiramatsu, Matsubara, Fujita, Sullivan, & Hara, 2006). In some marine teleosts that spawn pelagic eggs, these yolk proteins are further cleaved in oocytes and supply a pool of free amino acids (i.e., diffusible nutrients and osmotic effectors) during ovarian follicle maturation, whereas this thorough proteolysis does not occur in freshwater species such as salmonids (Hiramatsu, Cheek, Sullivan, Matsubara, & Hara, 2005; Hiramatsu et al., 2006). Additionally, in both cases, some or all yolk proteins are not fully digested during follicle maturation; researchers have found that some β′-c exists in the ovulated egg (Amano et al., 2007; Hiramatsu et al., 2002), suggesting that β′-c remains in the yolk during the early cleavage stage of the embryo. It is known that the enzymatic cleavage of the yolk proteins described above is caused by cathepsin families (Cavalli, Kashiwagi, & Iwai, 1997; Hiramatsu et al., 2002; Imamura, Yabu, & Yamashita, 2008; Raldua, Fabra, Bozzo, Weber, & Cerda, 2006). Therefore, the structure of β′-c seems to be stable to proteolysis during development of fish embryo. However, there is no information about gastrointestinal digestion of β′-c. Investigation of the relationship between the digestion behaviour of β′-c and its IgE-reactivity is important for understanding overview of fish roe allergy.

* Corresponding author. Tel./fax: +81 138 40 5516.
E-mail address: saeki@fish.hokudai.ac.jp (H. Saeki).

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High proteolytic resistance is one of the important characteristics of food-allergen proteins (Bannon, Fu, Kimber, & Hinton, 2003; Besler, Steinhut, & Paschke, 2001; Untersmayr & Jensen-Jarolim, 2006). For example, food allergens such as ovomucoid in egg white (Kovacs-Nolan, Zhang, Hayakawa, & Mine, 2000), β-lactoalbumin in cow milk (Astwood, Leach, & Fuchs, 1996), trypsin inhibitor in peanut (Ara h 2) (Lehmann et al., 2006), lipid transfer protein in grape (Vassilopoulou et al., 2006) and β-conglycininin soybean (Astwood et al., 1996), actinidin in kiwifruit (Bublin et al., 2008), and tropomyosin in crab (Liu et al., 2010) have high stability to digestion by gastrointestinal enzymes. The authors found that all the sera of salmon-roe-allergic patients ($n = 20$) contained a specific IgE that showed a strong reaction to β′-c, suggesting β′-c as a common major allergen in salmon roe allergy (Shimizu et al., 2009). Thus, the proteolytic resistance of β′-c may contribute to its high allergenicity in the case of fish roe allergy. However, little information about the structure of salmonid β′-c has been published.

The objective of this work was to estimate the effect of proteolysis on the allergenicity of chum salmon β′-c. Changes in the IgE-binding ability of β′-c during pepsin and trypsin digestion were investigated by monitoring its proteolytic cleavage. β′-c that degraded the subunit structure by carboxymethylation was also examined in order to understand the involvement of a tertiary structure in the proteolytic resistance of IgE-binding ability. Additionally, the structure of β′-c in terms of its contribution to proteolytic resistance was studied by analyzing amino acid sequences of the digestion fragments.

2. Materials and methods

2.1. Fish roe

Fresh chum salmon (*Oncorhyncus keta*) roe was purchased at a local fish market. It was washed with 0.16 M NaCl and stored at −60 °C until use.

2.2. Sera of fish-roe-allergic patients

Sera from 13 patients diagnosed with salmon roe allergy were selected for this study (age range, 5 months–12 years). Sera from two nonallergic individuals (age, 31 and 50 years) were also used as the control. Table 1 contains the clinical information of the patients. Each serum was subjected to capsulated hydrophilic carrier polymer–radio allergosorbent test (CAP–RAST) (Sampson & Ho, 1997) to determine the total IgE and specific IgE levels for chum salmon roe allergy. In the diagnostic system (ImmunoCAP, Phadia AB, Uppsala, Sweden), the whole extract of chum salmon roe was used as a solid-phase antigen. CAP–RAST score was determined from 0 (negative) to 6 (strong positive), according to the level of the specific IgE concentration. All patients' sera were evaluated as "positive". The patients' sera were frozen at <−60 °C for 2–12 months and were then thawed and mixed with the same volume of Dulbecco's phosphate buffered saline (pH 7.5; PBS) containing 0.2% NaN_3. They were stored at 4 °C until use.

2.3. IgG antibody against β′-c

The purified chum salmon β′-c was emulsified with Freund's incomplete adjuvant (Pierce, Rockford, IL). The emulsions were injected into rabbits (New Zealand White, male, 3 months old) once a week for 4 weeks. One week after the fourth injection, rabbit blood was gathered and centrifuged at 3000 g for 15 min to collect the supernatant. Forty per cent of saturated ammonium sulphate at the final concentration was added to the supernatant, and the mixture was centrifuged at 30,000 g for 30 min. The supernatant was dialysed against PBS and diluted with the same volume of PBS containing 0.2% NaN_3. The antibody against β′-c (anti-β′) thus obtained was stored at 5 °C until use. The animal experiment was performed according to the Guidelines Concerning Animal Experiments at Hokkaido University.

2.4. Preparation of yolk protein

Yolk protein extract (YPE) and β′-c were prepared from chum salmon roe by the method of Hiramatsu and Hara (1996), with a slight modification. Briefly, the roe was homogenised in 2-fold weight of 0.5 M NaCl containing 20 mM Tris–HCl (pH 8.0) using a potter homogenizer. The salt-soluble extract was centrifuged at 2000 g for 15 min to remove the floating oil layer and was further centrifuged at 20,000 g for 30 min. The supernatant was dropped into a 10-fold volume of cold distilled water, and the precipitate generated in this step was collected by centrifugation at 20,000 g for 30 min and dissolved in 0.5 M NaCl (pH 8.0). The fraction used in this study was YPE. β′-c was prepared from the YPE thus obtained. Sixty-seven per cent of saturated ammonium sulphate at the final concentration was added to the YPE. After centrifugation at 15,000 g for 30 min, the precipitate was redissolved in 0.5 M NaCl (pH 8.0) and loaded onto a Sephacryl S-200HR column (ø1.6 × 60 cm, GE Healthcare, Piscataway, NJ) to purify the β′-c.

Table 1
List of allergic patients hypersensitive to chum salmon roe.

Serum	Sex	Age (year)	CAP–RAST		Hypersensitivity reaction
			Total IgE (lU/mL)	Specific IgE (UA/mL, class)	
P1	F	2	349	86.8 (5)	AD
P2	M	11 Months	538	68.1 (5)	AD
P3	M	1	524	90.0 (5)	AD
P4	F	5 Months	24	5.1 (3)	AD, Ur
P5	M	2	9120	42.0 (4)	AD
P6	F	1	668	76.6 (5)	AD
P7	M	2	2398	66.9 (5)	AD, Ur
P8	F	11	3452	>100 (6)	AD
P9	M	5	5210	29.8 (4)	AD, BA
P10	M	2	2398	66.9 (5)	AD, Ur
P11	M	3	756	>100 (6)	AD, BA
P12	M	6	2936	>100 (6)	Ur
P13	M	3	402	63.3 (5)	AD
C1	M	31	–	–	–
C2	M	50	–	–	–

AD, atopic dermatitis; BA, bronchial asthma; Ur, urticaria; –, no data.