

INVESTIGATIVE REPORT

Identification of a Major Yolk Protein as an Allergen in Sea Urchin Roe

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Anaphylaxis after eating sea urchin roe has been reported. However, its major allergens have not yet been identified. The aim of this study was to identify the major allergens of sea urchin roe. Proteins of sea urchin roe were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis and two-dimensional electrophoresis (2-DE). An immunoglobulin (Ig)E-binding protein was detected by immunoblotting using the patient's serum. An allergen isolated from 2DE-gel was identified by peptide mass fingerprinting using matrix-assisted laser desorption/ionization-time of flight-mass spectrometry. Immunoblot analysis of sea urchin extracts showed that a 160-kDa protein at pI 6–7 was recognized by the patient's IgE. Peptide mass fingerprint analysis revealed that the protein was the major yolk protein (152 kDa, pI 6.9) of sea urchins. The results show that a major allergen of sea urchin roe is the major yolk protein. *Key words: sea urchin roe; anaphylaxis; major yolk protein; allergen.*

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Sea urchins are members of a large group of marine invertebrates in the phylum Echinodermata, subphylum Echinozoa, class Echinoidea. Their shell is globular in shape with sharp spines (1). Skin reactions to sea urchin stings have been described (2). Sea urchin roe is a popular food in Korea and Japan, and is called “*uni*” in Japanese sushi cuisine. Among the 800 species of sea urchin, there are 16 edible species, including *Strogolocentrotus intermedius*, *S. nudus* and *Pseudocentrotus depressus*, and the edible part is the gonads (testis and ovary). Worldwide consumption of sea urchin has been increasing with the globalization of food culture. There have been only a few reports of anaphylaxis after eating sea urchin roe (raw and boiled) (1, 3, 4). One allergen reported so far is a 118-kDa protein, which has not yet been identified (1, 4). The aim of this study was to identify the major allergens of sea urchin roe.

METHODS

Patient

A 26-year-old woman with no atopic diseases noticed swelling of her lips soon after starting to eat dinner. Within 30 min, eyelid swelling, rhinostenosis and generalized itching appeared. The patient was evaluated in the emergency department of our hospital. Physical examination revealed mild distress, blood pressure of 119/60 mmHg, pulse rate of 87/min, oxygen saturation of 93%, face swelling, rhinostenosis, and generalized whealing. She was treated immediately with hydrocortisone (Saxizon®, 300 mg i.v.), chlorpheniramine (Polaramine®, 20 mg by i.v. injection) and adrenaline (Bosmin®, 0.3 ml by i.m. injection). Symptoms disappeared within a few hours. She had eaten sashimi, cup steamed egg custard, raw oyster, and food served in a pot for dinner and had also drunk beer. Sea urchin roe was served with other pieces of sashimi on some plates. At the age of 13 years, the patient had had an episode of allergic reactions similar to this episode after consuming sea urchin roe as sushi. She had recovered after several hours without specific therapy. She did not eat sea urchin roe thereafter. The sushi meal consisted of sea urchin roe, dried pressed laver and vinegared rice. We suspected that the episode was caused by sea urchin food allergy.

Sample preparation

Sea urchin (*Echinina*), commonly available in markets in Japan, was used for the analysis. Sea urchin roe was disrupted in physiological saline using TissueLyser (QIAGEN, Valencia, WA, USA). The resulting lysate was centrifuged at 20,000 × g for 15 min at 4°C and the supernatant was used as a soluble fraction. The pellet was solubilized in 10% sodium dodecyl sulphate solution and used as an insoluble fraction. The samples were divided into aliquots and stored at –20°C.

Skin prick testing

The sea urchin roe-lysate was diluted with physiological saline and used for skin prick testing. For the prick-to-prick test, fresh sea urchin roe was used. Histamine dihydrochloride (10 mg/ml) and Allergen Scratch Extract Torii Control Solution, an aqueous solution containing 50% glycerol and 5% NaCl (Torii & Co., Ltd, Tokyo, Japan), were used as positive and negative controls, respectively.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis and IgE immunoblotting

Isolated proteins were separated by 7.5% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), as described by Laemmli (5), under reducing conditions with

2% mercaptoethanol. Separated proteins were stained with Coomassie Brilliant Blue R-250 (CBB). For immunoblotting, proteins were electrophoretically transferred to a polyvinylidene difluoride membrane (Millipore, Bedford, MA, USA) and blocked with 1% bovine serum albumin (BSA) in TBST (50 mM Tris-buffered saline containing 0.1% Tween-20, pH 7.6). The membrane was washed with TBST (Promega, Madison, WI, USA) and incubated with a 1:10 dilution of the patient's serum in TBST containing 1% BSA. After washing with TBST, membranes were incubated with peroxidase-labelled goat anti-human IgE antibodies (Biosource, Camarillo, CA, USA) for 1 h at room temperature. Human IgE was detected with an ECL Plus Western Blotting Detection System (GE Healthcare BioSciences, LittleChalfont, UK) and visualized on X-ray film. As a negative control, serum of a healthy subject without atopic disease was used.

Two-dimensional electrophoresis

Proteins in the soluble fraction were precipitated with two volumes of acetone on ice and then solubilized in two-dimensional electrophoresis (2-DE) sample buffer containing 6 M urea, 1 M thiourea, 3% CHAPS, 1% Triton-X100, 0.1 M iodoacetamide and 60 mM Tris-HCl (pH 8.9). Protein concentration in the 2-DE sample buffer was determined by using a DC/RC protein assay kit (Bio-Rad, Hercules, CA, USA). Protein (250 µg) was applied at the cathodic end of the agarose isoelectric focusing (IEF) gel (pH 3–10, 75 mm × 2.5 ID, ATTO), and an overlaying solution containing 4 M urea, 1 M thiourea and 1% arginine hydrochloride was filled above the sample solution. First-dimensional separation was performed with an AE-6540MM electrophoresis apparatus (ATTO) by using 0.2 M NaOH solution and 10 mM phosphate solution as the catholyte and anolyte, respectively. The following running conditions were used: 100 V for 15 min, 200 V for 15 min, and 300 V for 3 h 15 min. IEF gels were fixed in 10% trichloroacetic acid (TCA) solution for 1 h and 1% TCA solution for 1 h. The fixed first-dimensional IEF gel was equilibrated in a solution containing 50 mM Tris-HCl (pH 6.8), 2% SDS and bromophenol blue and then applied to 7.5% SDS-PAGE for secondary separation. For 2-DE immunoblotting, proteins in the 2-DE gel were transferred to a polyvinylidene difluoride membrane. The IgE-binding protein separated by 2-DE was detected with an ECL Plus kit, as described above.

Matrix-assisted laser desorption/ionization-time of flight-mass spectrometry analysis and database searching

The IgE-binding protein was excised from the 2DE gel, stained with CBB, and digested using ProteasMAX™ Surfactant (Promega) and Trypsin Gold (Promega) following the manufacturer's instructions. The peptide solutions were desalted with ZipTip (Millipore) and then mixed with the same volume of α-cyano-4-hydroxycinnamic acid (10 mg/ml in 50% acetonitrile/0.1% TFA) on a MALDI sample plate (Wako, Osaka, Japan). Peptide mass spectra were recorded on a matrix-assisted laser desorption/ionization-time of flight-mass spectrometry (MALDI-TOF/MS) spectrometer Voyager DE PRO (PerSeptive biosystems, Framingham, MA, USA) equipped with delayed extraction. The spectra were obtained in reflectron mode at 25 kV acceleration voltage, 74% grid voltage, 0% wire voltage, 100 ns delay time, and low mass gate at 800 m/z. An external calibration with three peptides (angiotensin II, P14R, ACTH fragment (18–39)) covering a 950–2500 Da mass range was used during measurement. A search was carried out for monoisotopic against silico digests from all species in the UniProtKB/SwissProt database (release 56.9) using Aldente servers (<http://www.expasy.org>).

RESULTS

Skin prick testing

The prick-to-prick test with sea urchin roe was strongly positive (27 × 16 mm wheal and 65 × 40 mm flare), and the skin prick test with the sea urchin roe-lysate also showed positive reaction (15 × 9 mm wheal and 40 × 30 mm flare) (Fig. 1). Two healthy subjects had negative reactions in these tests (data not shown).

Detection of IgE reacting to sea urchin roe extracts in the patient's serum

Proteins in sea urchin roe were fractioned by their solubility for physiological saline, and both soluble and insoluble fractions were separated by SDS-PAGE. Immunoblot analysis showed that the patient's serum IgE reacted to a 160-kDa protein in the soluble fraction, whereas no IgE reactivity was observed in the healthy subject (Fig. 2).

Two-dimensional electrophoresis immunoblotting and identification of allergen

The soluble fraction of sea urchin roe-lysate was separated using 2-DE. Immunoblot analysis of the 2-DE showed a reaction of IgE in the patient's serum to a 160-kDa protein at pI 6–7 (Fig. 3). The IgE-binding 160-kDa protein detected by 2-DE immunoblotting was analysed by MALDI-TOF/MS. A tryptic peptide

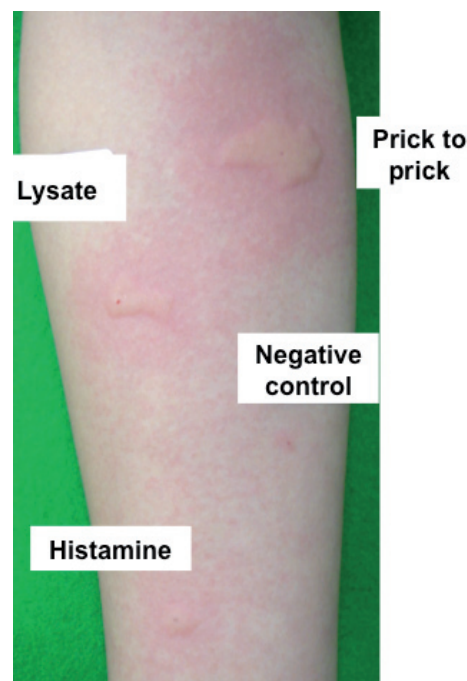


Fig. 1. Skin prick test was performed on the arm in prick-to-prick fashion using diluted lysate of sea urchin roe. The skin reaction was evaluated 15 min after testing.

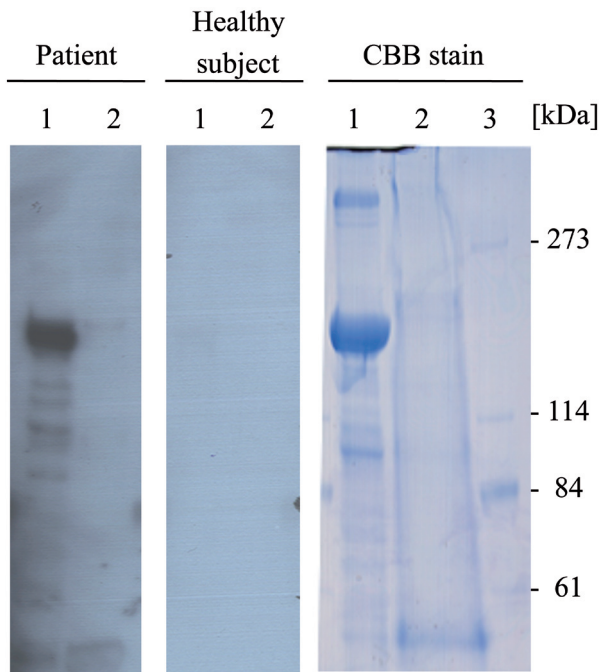


Fig. 2. Immunoglobulin (Ig)E immunoblotting using sea urchin lysates. Proteins in the soluble (lane 1) and the insoluble fraction (lane 2) were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis. The IgE reactivity was analysed by immunoblot using sera of the patient and a healthy subject. Total proteins and molecular standard (lane 3) were stained with Coomassie Brilliant Blue R-250 (CBB).

mixture of the protein revealed 43 mass peaks by MALDI-TOF/MS, and the results were analysed by peptide mass fingerprinting. The protein was identified by peptide mass fingerprint analysis as the major yolk protein (MYP, 152 kDa, pI 6.9) of *S. purpuratus* (accession number P19615) with 11% sequence coverage and 32 ppm of maximum mass differences.

DISCUSSION

We report here a case of anaphylaxis in response to sea urchin roe, in which we identified MYP as the causative allergen. The strong positive skin prick test reaction and a previous allergy episode suggested that the patient had a food allergy to sea urchin roe. Despite the increasing

consumption of sea urchin roe all over the world, few cases of sea urchin roe allergy have been reported so far. Rodriguez et al. (1) and Damiani et al. (4) have determined by immunoblot analysis that one allergen is a 118-kDa protein, but the allergens have not yet been identified. In our study, a major allergen of sea urchin roe, a protein observed at 160 kDa in SDS-PAGE, was identified as a MYP using mass spectrometry. Besides MYP, we detected some minor allergens with weak IgE-binding reactivity in the 100–140 kDa range. The previously reported 118-kDa protein may be one of the allergens that we detected as minor allergens.

MYP, a glycoprotein of 170 kDa, was originally identified as the predominant component of yolk granules in sea urchin eggs. It is an essential nutrition for embryogenesis (6). In sea urchins, MYP is also contained in the coelomic fluid and nutritive phagocytes of the gonad in both sexes in contrast to other oviparous animals, in which yolk protein is female-specific. MYP in the coelomic fluid has a higher molecular mass (180 kDa) than that in the gonad (170 kDa) (6, 7). Unuma et al. (8) proposed that MYP in coelomic fluid is synthesized in the digestive tract, binds zinc derived from food, transports it to the gonad through the coelomic fluid, and is modified to a 170-kDa MYP in the gonad.

In this anaphylaxis episode, the patient did not eat sea urchin roe directly. The allergic reaction was probably induced by an extremely small quantity of allergen that had adhered to other food on the plate or to chopsticks. This speculation is reasonable considering that MYP is abundant in the edible part of sea urchin roe.

Sea urchins are not closely related to fin fish, molluscs or crustaceans. Sea urchins are spiny sea creatures of the class *Echinoidea*, which includes starfish and sea cucumbers (3). Sea cucumbers have been eaten as a delicacy in Japan and China for more than 1000 years, and the ovaries have been valued highly as “Kuchiko” or “Konoko”. Eggs of the starfish are also cooked in one region of Japan. The present patient has had no episode of allergic reaction after eating spawns. Since she has never eaten sea cucumbers or starfish, it is not known whether the patient has a clinical allergy for sea cucumber or starfish. There have been no reports of

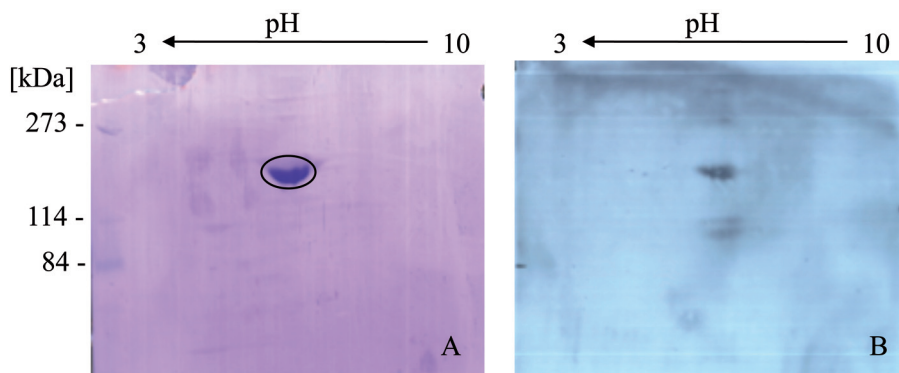


Fig. 3. Two-dimensional electrophoresis immunoblotting. The soluble protein from sea urchin roe was separated by two-dimensional electrophoresis (2-DE) and stained with Coomassie Brilliant Blue R-250 (CBB). (A) A 160-kDa protein was recognized with the patient's IgE. (B) Arrows point to vertical streaks at the side of the second-dimensional sodium dodecyl sulphate (SDS)-gel. The circle indicates the protein analysed by peptide mass fingerprinting.

material causing cross-reaction with sea urchin. MYP contained in the sea urchin coelomic fluid was formally called sea urchin vitellogenin based on its similarity to vertebrate vitellogenin (yolk protein precursor) in the biological features as a yolk material (9). However, whole sequencing of cDNA encoding sea urchin vitellogenin revealed that it is not homologous to vertebrate vitellogenin at all (10). Instead, MYP has a small, but seemingly genuine, homology to vertebrate transferrin, a blood plasma protein concerned with iron transport. A lower homology of MYP to vertebrate vitellogenin suggests that IgE-binding epitopes of MYP are not present in vertebrate vitellogenin. We considered these to be reasons why the patient has episodes of allergic reaction to sea urchin roe but not to spawns.

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