Seafood allergy and allergens
School of Internet
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Food allergy is type I immediate hypersensitivity

Food allergy, together with pollen and mite allergies, is a representative of type I immediate hypersensitivity mediated by immunoglobulin E (IgE) antibodies.

Allergic people tend to produce IgE.
What is allergen?

Allergens:
I. are proteins.
II. are non-toxic to healthy people.
III. cause allergic symptoms in sensitized subjects.
IV. can bind to IgE antibodies on mast cells distributed through the surface of body and digestive tract.
V. have IgE-binding epitopes which are important for the onset of allergic symptoms.
Although food allergy has less number of patients than other type 1 allergies such as pollen and mite allergies, it is of particular importance in the following points:

1) Its causes are foods which are indispensable in maintaining human life.
   
   Some patients with allergies for various foods are troubled with supplementation.

2) It is sometimes fatal due to anaphylactic shock (systemic allergic reaction).
Seafood allergy is a serious problem in countries where a lot of seafood is consumed.
Countermeasure against food allergy in Japan
(food allergen labeling system)

April 1, 2001: A food allergen labeling system was mandated for the first time in the world. The system included 5 specific ingredients (mandatory for labeling) and 19 subspecific ingredients (recommended for labeling).

November 16, 2004: Banana was additionally selected as a subspecific ingredient.

July 3, 2008: Shrimp/prawn and crab were upgraded to specific ingredients.

<table>
<thead>
<tr>
<th>Labeling</th>
<th>Ingredients</th>
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<tbody>
<tr>
<td>Mandatory (specific ingredients)</td>
<td><strong>Shrimp/prawn, crab</strong>, wheat, buckwheat, egg, milk/milk products, peanut (7 items)</td>
</tr>
<tr>
<td>Recommended (subspecific ingredients)</td>
<td><strong>Abalone, squid, salmon roe</strong>, orange, kiwifruit, beef, walnut, <strong>salmon, mackerel</strong>, soybean, chicken, banana, pork, matsutake mushroom, peach, yam, apple, gelatin (18 items)</td>
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<tr>
<td>Year</td>
<td>Event</td>
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<tr>
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<tr>
<td>1966</td>
<td><strong>Discovery of IgE antibodies</strong> (Dr. Kimishige Ishizaka: Johns Hopkins University)</td>
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<tr>
<td>1975</td>
<td>The major allergen (Gad c 1) from codfish (<em>Gadus callarias</em>) in Northern Europe was demonstrated to be parvalbumin. Gad c 1 is the first allergen identified at the molecular level.</td>
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<tr>
<td>1983</td>
<td>Analysis of sequential IgE-binding epitopes of Gad c 1</td>
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<tr>
<td>Early 1990’s</td>
<td>Tropomyosin was identified as the major allergen in 3 shrimp/prawns (Indian prawn, brown shrimp, offshore greasyback prawn). There has been no information about fish allergens except for Gad c 1 and molluscan allergens.</td>
</tr>
<tr>
<td>Mid-1990’s – present</td>
<td>Molecular studies on allergens from fish, crustaceans and molluscs (squid, octopus, gastropod and bivalve) have greatly developed.</td>
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## Seafood allergens so far identified

<table>
<thead>
<tr>
<th>Seafood</th>
<th>Major allergen</th>
<th>Other allergens</th>
</tr>
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<tbody>
<tr>
<td>Fish</td>
<td>Parvalbumin</td>
<td>Collagen, aldehyde phosphate dehydrogenase, transferrin</td>
</tr>
<tr>
<td>Crustacean (shrimp, prawn, crab)</td>
<td>Tropomyosin, sarcoplasmic calcium-binding protein (SCP)</td>
<td>Arginine kinase, myosin light chain, hemocyanin</td>
</tr>
<tr>
<td>Mollusc (squid, octopus, gastropod, bivalve)</td>
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# Seafood allergy and allergens

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<td>Paramyosin</td>
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</table>
What is parvalbumin?

- Sarcoplasmic protein of 12 kDa
- \( \text{Ca}^{2+} \)-binding protein containing 3 EF-hand motifs (AB, CD and EF domains) that are characterized by the \( \alpha \)-helix-loop-\( \alpha \)-helix structure (\( \text{Ca}^{2+} \)-binding sites are CD and EF domains)
- Involvement in the process of muscle relaxation.
- Vertebrate-specific protein, contained at especially high concentrations in the muscle of fish and amphibians.
- Its allergenicity is considerably heat-stable.
Contents of parvalbumin in white and dark muscles of fish

★ Contents of parvalbumin in the white muscle is higher than those in the dark muscle. Moreover, parvalbumin in the white muscle is identical with that in the dark muscle.

→ The fish dark muscle is less allergenic than the white muscle.

★ Contents of parvalbumin vary widely from fish to fish.

→ Allergenicity might vary among fish species.

Contents of parvalbumin in various parts of fish

Japanese jack mackerel

- Dark muscle
- Ventral part
- Caudal part
- White muscle
- Dorsal part
- Rostral part

Bar chart showing the contents of parvalbumin with values:
- Dark muscle: 7.4
- Ventral part: 7.6
- Caudal part: 5.2
- White muscle: 3.5
- Dorsal part: 2.7
- Rostral part: 2.9
Contents of parvalbumin vary widely among fish species.
Contents of parvalbumin in various fish

- Splendid alfonsino
- Goldeye rockfish
- Red barracuda
- Flying fish
- Rosy seabass
- Red sea bream
- Chicken grunt
- Round herring
- Rainbow trout
- Pacific saury
- Japanese sardine
- White croaker
- Blue mackerel
- Atlantic salmon
- Chub mackerel
- Chum salmon
- Skipjack tuna
- Silver salmon
- Swordfish
- Yellowfin tuna
- Bigeye tuna

Contents of parvalbumin: migratory fish < rockfish
big fish < small fish
IgE-reactivity of patients against various fish

Allergenicity is proportional to contents of parvalbumin.
IgE-reactivity depends on Ca\(^{2+}\) widely regardless of fish species or patients. → Conformational epitopes are critical.
Conformational IgE-binding epitopes of parvalbumin from Chub mackerel

ELISA using modified parvalbumin, each amino acid residue of which was substituted to Ala, elucidated two conformational IgE-binding epitopes (unpublished).
Seafood allergy and allergens

Fish
- Parvalbumin
- Collagen

Crustaceans
- Tropomyosin
- Sarcoplasmic calcium-binding protein (SCP)

Mollusks
- Tropomyosin
- Paramyosin
What is collagen?

★ Collagen is a stroma protein which is composed of 3 α chains (molecular masse of each chain is about 100 kDa).

Mammals: \((\alpha 1)_2\alpha 2\)  
Fish: \((\alpha 1)_2\alpha 2\) or \(\alpha 1\alpha 2\alpha 3\)

★ Has repeats of the sequence of Gly-X-Y.

★ Most of Pro and Lys are hydroxylated.

★ Raveled and fractionated when heated. Raveled or fractionated collagen is called gelatin.

★ Forms ligament, dermis or cartilage and is contained universally in animals.
 Importance of collagen as fish allergen and cross-reactivity with mammalian collagen

Fish collagen is recognized by one third of fish-allergic patients. It’s has cross-reactivity.

→ collagen is an important fish allergen.

There is no cross-reactivity between fish and mammalian collagens.

Analysis of IgE-binding epitope of collagen α2 chain from rainbow trout

1) Analysis of IgE reactivity of overlapping recombinant proteins spanning the entire sequence of mature collagen.

2) Analysis of IgE reactivity of overlapping peptides spanning a segment (R5 protein) containing a major IgE-binding epitope.

A major IgE-binding epitope exists in the region 941-960. Amino acid sequence of this region is significantly conserved among collagen α2 chains from other fish. This suggests that this region is a common IgE-binding epitope of collagen α2 chain from various fish. On the other hand, this sequence widely varies compared with collagen α2 chain from mammals.

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What is tropomyosin?

★ Tropomyosin (70 kDa) is a myofibrillar salt-soluble protein. It constitutes a myofilament with actin and troponin which is involved in the muscle contraction.

★ Tropomyosin is a homodimer. Two subunits entwine with each other and this structure is called coiled coil structure. Each subunit has a repetitive sequence composed of 7 amino acid residues.

★ It is contained in animals universally.

★ Allergenicity of tropomyosin is heat-stable.
Cross-reactivity of tropomyosin among various animals

- **Crustaceans**
  - prawn/shrimp
  - crab
  - other crustaceans
- **Land-dwelling arthropods**
  - (mites, cockroach etc.)
- **Mollusks**
  - squid, octopus
  - gastropod, bivalve
- **Nematode worms**
  - (Ascaris, Anisakis etc.,)

Fish and Mammals do not cross-react with Crustaceans, but other crustaceans can cross-react with each other.

- **Tropomyosin is a pan-allergen of invertebrates.**
- **Invertebrate tropomyosins have cross-reactivity with each other but no cross-reactivity with vertebrate tropomyosins.**
Common allergen of crustaceans is tropomyosin

SDS-PAGE

IgE-immunoblotting

Tropomyosin purified from American lobster, Black tiger prawn, Kuruma prawn, Alaskan pink shrimp, Red king crab (leg muscle), snow crab (leg muscle), horsehair crab (chest protection muscle), Red king crab (chest protection muscle), snow crab (chest protection muscle), horsehair crab (leg muscle)

Prawn/shrimp Crab Prawn/shrimp Crab

Common allergen of cephalopods is tropomyosin

Common allergen of gastropods and bivalves is tropomyosin

![Graph showing IgE-ELISA and IgE-immunoblotting results for various seafood types](image)

- Disc abalone
- Turban shell
- Whelk
- Middendorf's buccinum
- Blood cockle
- Japanese cockle
- Japanese oyster
- Surf clam
- Horse clam
- Razor clam
- Short-neck clam
- American lobster
- Tropomyosin purified from turban shell

Amino acid sequences of crustacean tropomyosins

In order from the top; brown shrimp (fast), black tiger prawn (fast), kuruma prawn (fast), Alaskan pink shrimp (fast), American lobster (fast), American lobster (slow-twitch), American lobster (slow- tonic), red king crab (fast), red king crab (slow-tonic), snow crab (slow- tonic), horsehair crab (slow- twitch), horsehair crab (slow- tonic). ■: residues differing from brown shrimp

Sequence identities are very high (over 90%). The IgE-binding epitopes proposed for brown shrimp tropomyosin are also conserved well among crustacean tropomyosins.

Sequence identities between crustacean and molluscan tropomyosins are about only 60%. The IgE-binding epitopes proposed for brown shrimp tropomyosin are less conserved among molluscan tropomyosins.

## Seafood allergy and allergens

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IgE-reactivity of SCP, tropomyosin and arginine kinase purified from black tiger prawn

A half of 16 crustacean-allergic patients recognize SCP.
→ Is SCP a major allergen?

13 of these 16 patients react against tropomyosin.
→ There is no doubt that tropomyosin is a major allergen.

Reactivity against arginine kinase is very low.
→ Arginine kinase is not an important allergen.

Of these patients, there are two SCP-specific patients.

Shiomi et al. Int Arch Allergy Immunol 146, 91-98 (2008)
SCP is prawn/shrimp-specific allergen

1, Purified SCP; 2, purified tropomyosin; 3, black tiger prawn; 4, kuruma prawn; 5, American lobster; 6, Alaskan pink shrimp; 7, red king crab; 8, snow crab

★ IgE-reactivity of SCP:
  kuruma prawns (++) ; other prawns/shrimps (+); crabs (-); mollusks (-)

★ Some crustacean-allergic patients react only to prawns and shrimp.

Prawn/shrimp-specific patients might recognize SCP.

Shiomi et al. Int Arch Allergy Immunol 146, 91-98 (2008)
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A 100 kDa allergen was purified from disc abalone and identified as paramyosin. Paramyosin is an invertebrate-specific protein and forms a myofilament with myosin.
IgE-reactivity of paramyosins from various crustaceans and mollusks

Shellfish (bivalve, gastropod)  Cephalopod  Prawn crab
Of 18 crustacean and molluscan-allergic patients, 16 reacted against tropomyosin. → Tropomyosin is a major allergen.

The 16 patients who react against tropomyosin all reacted against paramyosin although the allergenicity of paramyosin is less than that of tropomyosin → Paramyosin is also a major allergen.

Subjects of future investigation relating to seafood allergens

For proper diagnosis, optimal medical treatment and protection on seafood allergy, we must accumulate more knowledge on seafood allergens.

Response to an incredible number of seafoods (e.g. “If a patient experienced fish allergy against one kind of fish, he should avoid eating all species of fish.”) Is this recommendation by doctor appropriate?

★ We must evaluate allergenicity of individual seafood based on quantity (contents) and quality (allergenicity).

Identification of unknown allergens

Analysis of primary structures of allergens and elucidation of their IgE-binding epitopes

Development of hypoallergenic seafoods