

Surimi and native codfish contain a common allergen identified as a 63-kDa protein

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We have compared the allergenicity of codfish and surimi (prepared from codfish) by skin testing, specific IgE-RIA, and leukocyte histamine release (LHR) in six fish-allergic patients. Prick tests were positive for codfish and, to a lesser extent, surimi. The percentages of labeled anti-IgE bound to surimi-Sepharose were $1.55 \pm 0.19\%$ and 3–6% with control and patient sera, respectively. Inhibition of the surimi protein-Sepharose IgE-RIA was greatest (80%) at protein concentrations of 13.4 and 408.5 $\mu\text{g/ml}$ for codfish and surimi extract, respectively. The allergenic protein was isolated by gel filtration and subjected to SDS-PAGE. The eluate from codfish contained several proteins ranging from 13 to 63 kDa, while the eluate from surimi contained a single 63-kDa protein. It was concluded that surimi contained a single allergenic protein.

Key words: food allergy; surimi.

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Food allergy to fish is frequent in well-developed countries, comprising 15.4% of food allergies in France and Switzerland (13, 19). In the pediatric population, fish is one of the most common sources of allergens in food allergy (7, 21). A broad range of food symptoms attributable to fish have been documented (1, 3, 15, 21). Fish allergy has been involved in asthma after inhalation of fish proteins (15). Fatal anaphylactic reactions induced by fish ingestion have also been described (23).

Codfish allergens were the first to be purified and characterized. Codfish contains one major allergen found in the sarcoplasmic proteins of white muscle. This allergen, *Gad c I* in the allergen nomenclature, is a parvalbumin (2, 8). Codfish also contains other intermediate and minor allergens (5, 6).

In the present study, we investigated the allergenicity of a Japanese food product named surimi, which is minced and thoroughly washed fish meat (11). Surimi is a mixture of small fish of many species. It lacks most soluble sarcoplasmic proteins, and is composed mainly of myofibrillar proteins (14). The production of surimi has greatly increased, and it uses cryoprotectors which slow the denaturation of frozen proteins (14). Surimi may have added starch, egg white, and other ingredients, forming an

elastic gel after cooking (11). Its applications in the food industry are numerous (14).

The modification of the balance between native proteins remaining in surimi suggested that this food-stuff might lack sarcoplasmic allergenic protein (11).

This study analyzes the characteristics of the allergenic proteins of surimi in gel filtration and electrophoresis, and demonstrates the allergenicity of a 63-kDa protein, by inhibition of specific IgE radioimmunoassay, with sera from six fish-allergic patients.

Material and methods

Patients

Thirteen patients (seven controls and six fish-allergic patients) were included in the study after giving informed consent. Inclusion criteria were obvious history of fish allergy with presence of specific IgE against codfish allergens, as shown by skin and radioallergosorbent test (RAST) (Phadebas). Controls (five females and two males, aged 14–68 years) had no history of food allergy and had negative skin and RAST reactivity to codfish. Two controls were atopic. The patient group (four females and two males) were aged 3–29 years. All were atopic. Clini-

serum with allergen ("negative control") and Cpmi is the radioactivity of material bound to the gel after preincubating the serum with soluble allergen.

Electrophoresis

The peaks collected from gel filtration were pooled, and the proteins were precipitated in the presence of chloroform and methanol, as described recently (10). After evaporation, the proteins were dissolved in 10 µl distilled water. SDS-PAGE of the proteins was performed with a 8–25% polyacrylamide gradient gel (Phast-System, Pharmacia, Uppsala, Sweden) in 0.20 mol/l Tris-HCl (pH 7.5) per 0.20 mol/l tricine per 0.55% SDS. The separation was done with 250 V (10.0 mA), at 15°C. Phosphorylase, albumin, ovalbumin, carbonic anhydrase, trypsin inhibitor, and α-lactalbumin were used as reference proteins. Each sample contained 2.5% for SDS and 5% for β-mercaptoethanol, and was heated at 100°C for 5 min. Proteins were visualized with the silver-staining method described by Heukeshoven & Dernick (12).

In vitro LHR tests were performed with whole heparinized blood, as described recently (17). Incubation of 200-µl aliquots was done for 30 min at 37°C with 100 µl of five dilutions from 10^{-1} to 10^{-5} of a 10-mg/ml solution of surimi. Codfish LHR tests were done with a solid support (codfish disk) instead of soluble allergen. The released histamine (H_i) was assayed in the supernatant which was collected after centrifugation at 800 g for 5 min at 4°C. The total blood histamine (H_t) was measured in a sixth tube to which distilled water was added. The blood cells were disrupted by freezing and unfreezing the sample. Basal histamine (H_o) was determined in a seventh tube in which 200 µl of blood was incubated with 1000 µl of buffer. The concentration of histamine in each tube was determined by commercial radioimmunoassay (Immunotech, Luminy, France). The LHR (%) was estimated by the following formula:

$$\text{LHR} = \frac{H_i - H_o}{H_t - H_o} \times 100$$

The test was considered to be positive when more than 10% of histamine was released.

Results

Prick tests were positive for codfish and, to a lesser extent, surimi in the allergic patients. Surimi diluted in sodium saline serum 0.9% gave a negative prick test in patients 3 and 4. The seven control subjects had negative prick tests.

The codfish RAST was positive in the six allergic patients. The percentage of ^{125}I -labeled anti-IgE bound to the disk varied from 15 to 43% (Fig. 1).

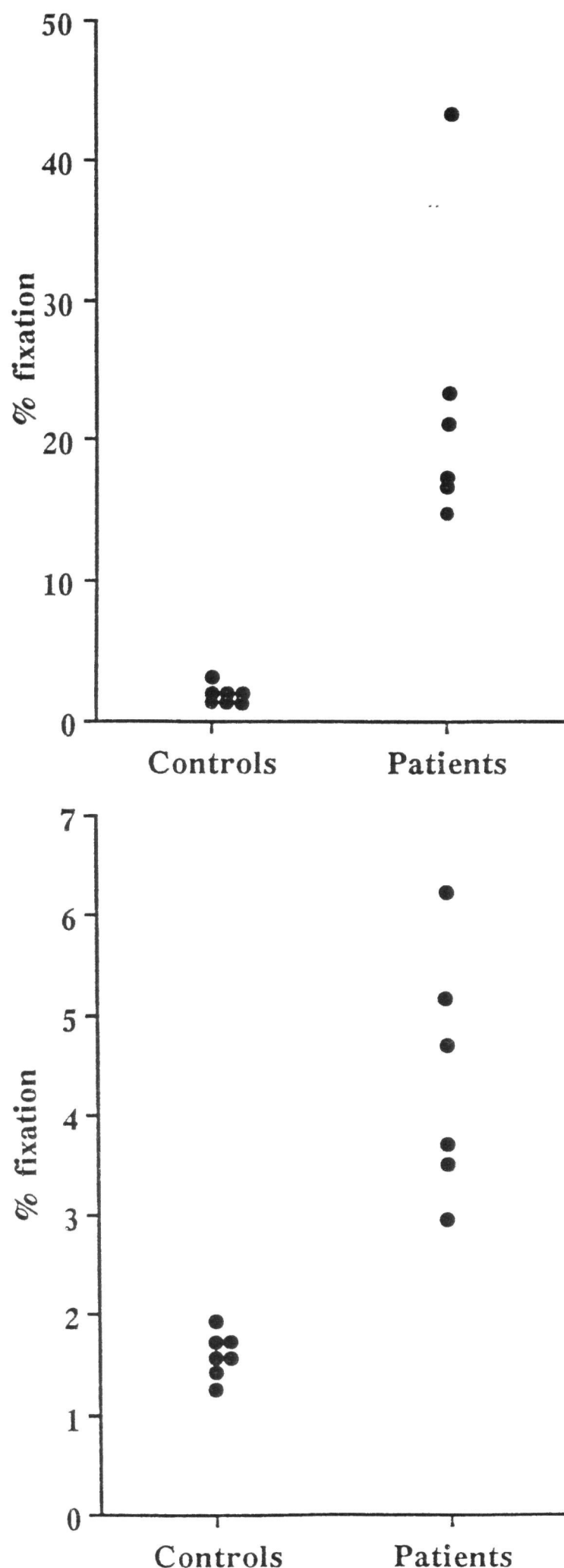


Fig. 1. RAST for codfish (top) and IgE-RIA with Sepharose-surimi (bottom) in six patients and seven controls. Results (y-axis) are expressed as percentage of labeled anti-IgE (^{125}I -iodinated IgG) fixed on solid reactive phase. Limit of normal uptake was estimated at 1.9% and 3.3%, respectively, for Sepharose-surimi-IgE-RIA and for RAST for codfish.

The percentage of labeled anti-IgE-bound surimi to protein-Sepharose was $1.55 \pm 0.19\%$ with the seven control sera. The upper limit of normal results was therefore estimated at 1.9% (mean + 2 SD). The surimi-IgE-RIA was higher than the upper normal limit in the six allergic patients, ranging from 3 to 6% (Fig. 1).

The codfish RAST was done in the presence of increasing amounts of soluble surimi for three patients. The inhibition of the codfish RAST with the solution of surimi proteins varied from 60 to 94% .

Inhibition of the surimi protein-Sepharose IgE-RIA was greatest at protein concentrations of 13.4 and $408.5 \mu\text{g/ml}$ for codfish and surimi extracts, respectively (Fig. 2). The inhibition percentage reached 80% with both allergens.

Absorbance at 280 nm of Superose 6 gel filtration eluate of codfish and surimi extracts detected two peaks for both samples, with nearly the same elution position. Absorbance of peak I was at 0.36 and 0.31 for surimi and codfish, respectively; it was at 0.02 and 0.25 for peak II from surimi and codfish, respectively. The K_d of the peaks were estimated at 0.30–0.40 (peak I) and 0.50–0.60 (peak II), corresponding, respectively, to mol. mass at 500–200 and 100–50 kDa. The maximum of the peak also corresponded to the maximum of inhibition of the surimi IgE-RIA by the eluted fractions, showing that both protein peaks contained the allergen (Fig. 3). Peak II was tested with electrophoresis. The codfish peak II was resolved in four bands with respective mol. mass of 13, 25, 37, and 63 kDa. It was concluded that the 13-, 25-, and 37-kDa proteins behaved as either aggregates or oligomers because they were eluted in the 100–50-kDa fractions in Superose 6 gel filtration. The surimi peak II was resolved as a single band with an estimated mol. mass of 63 kDa (Fig. 4).

The LHR tests were performed with soluble surimi and codfish on paper disks. The LHR was positive for both surimi and codfish (Table 1) in the six allergic subjects. It was negative for the seven control subjects.

Discussion

We have shown a reactivity for both codfish and surimi proteins in inhibition tests of IgE-RIA and in prick tests. Prick tests were positive with fish (commercial extract), natural codfish, and surimi. LHR tests were also positive for both surimi and codfish. Spontaneous histamine release was elevated for five of the six allergic subjects (Table 1). No release was obtained from the leukocytes of the seven control subjects, in assessment of the specificity of the histamine released from the leukocytes of the allergic subjects. Patients with food hypersensitivity and atopic dermatitis do have high spontaneous hista-

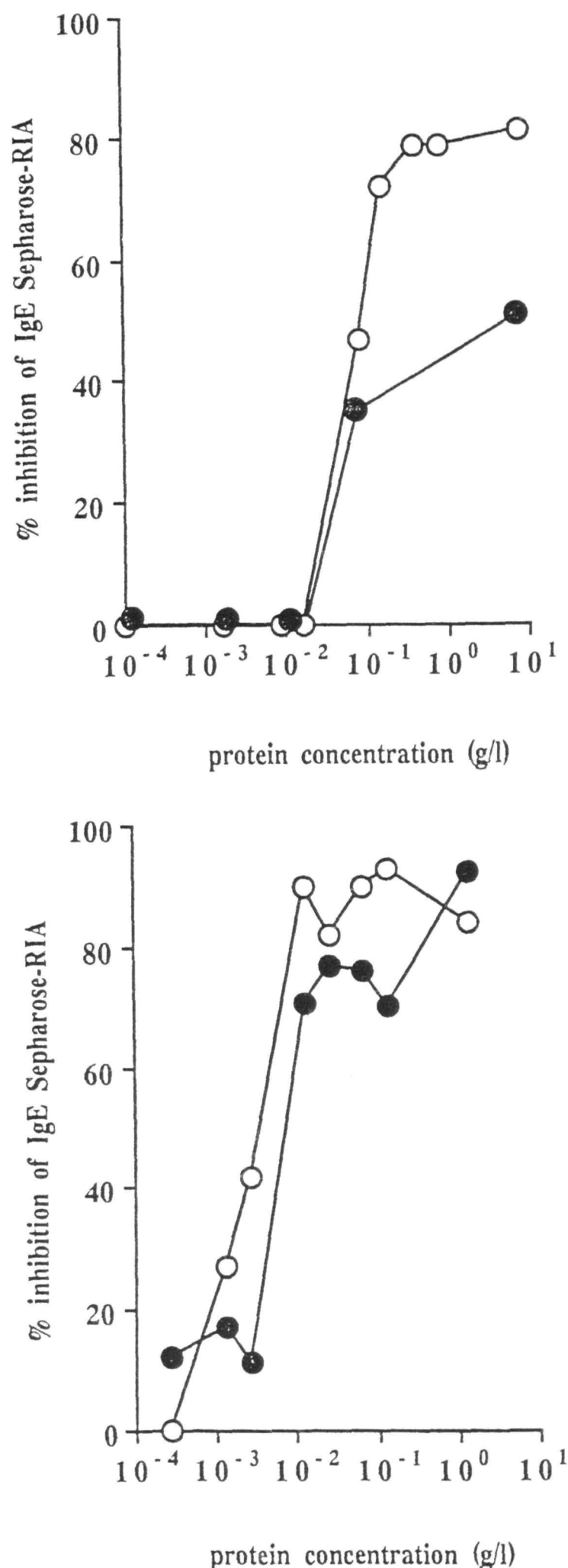


Fig. 2. Inhibition of surimi-Sepharose-IgE-RIA after incubation in presence of sera from patients 5 (○) and 6 (●) in presence of increasing concentration of either codfish (top) or surimi soluble protein extract (bottom). Maximal inhibition was about 80% with both allergen extracts. It was obtained at protein concentration 30-fold higher with codfish than with surimi extract.

Table 1. *In vitro* leukocyte histamine release (LHR) in presence of either surimi or codfish in six allergic patients and in seven controls

Patients (P) and controls (C)	Histamine values (nM)		Maximal LHR (%)	
	Whole blood h (H _t)	Basal histamine (H _o)	Surimi	Codfish
P1	648	67	30.1	18.5
P2	831	38	4.7	59.0
P3	597	54	35.5	17.5
P4	475	14	19.2	87.8
P5	392	145	59.1	38.4
P6	447	153.7	27.4	13.4
C1	356	11	5.5	3.2
C2	437	7	0.9	0
C3	206	5	0.5	0
C4	621	3	1.3	0.2
C5	368	12	0	0
C6	982	12	0.7	0.4
C7	581	10	0	0

mine release from peripheral blood basophils *in vitro* (18). The increased basophil releasability seems to be related to the histamine-releasing factor (HRF) produced by the mononuclear cells from food-allergic patients (16, 20). In our study the highest level of spontaneous histamine release was found in patient 6, who had atopic dermatitis.

Both codfish RAST and solid surimi-phase-IgE-RIA were positive in the allergic patients (Fig. 2). Inhibition of the uptake of ¹²⁵I-labeled anti-IgE by surimi protein-Sepharose was lower with surimi extract than with codfish extract; the greatest inhibition was obtained at protein concentrations of 408.5 and 13.4 µg/ml for surimi extract and codfish extract, respectively. This indicated a 30-fold increase in allergenic activity of codfish proteins.

The major allergen in codfish is a parvalbumin belonging to the sarcoplasmic proteins. It presents allergenic homology with proteins of various fish species (1–3). Its mol. mass is about 12.3 kDa according to Elsayed & Apold (8). The allergenic determinant is sequential, remaining unchanged after heating at 100°C for 10 min and after denaturation with several products. This allergenic determinant is composed of two closely connected carboxylic side-chains (Asp + Glu) kept at a critical distance from a lysine basic residue (-Asp-Glu-X-Lys). The region comprising residues 41–64 of allergen M encompasses three of the tetrapeptides described above (2, 8). The allergenicity is decreased by polymerization or by acetylation of the Tyr-30 residue, but these chemical modifications cannot be exploited commercially to reduce the allergenicity of codfish (4). The preparation of surimi includes an extensive washing process of myofibrillar proteins (14) which may eliminate most sarcoplasmic proteins. Theoretically, the myofibrillar protein fraction comprises the single proteins contained by this fish food product (22). In

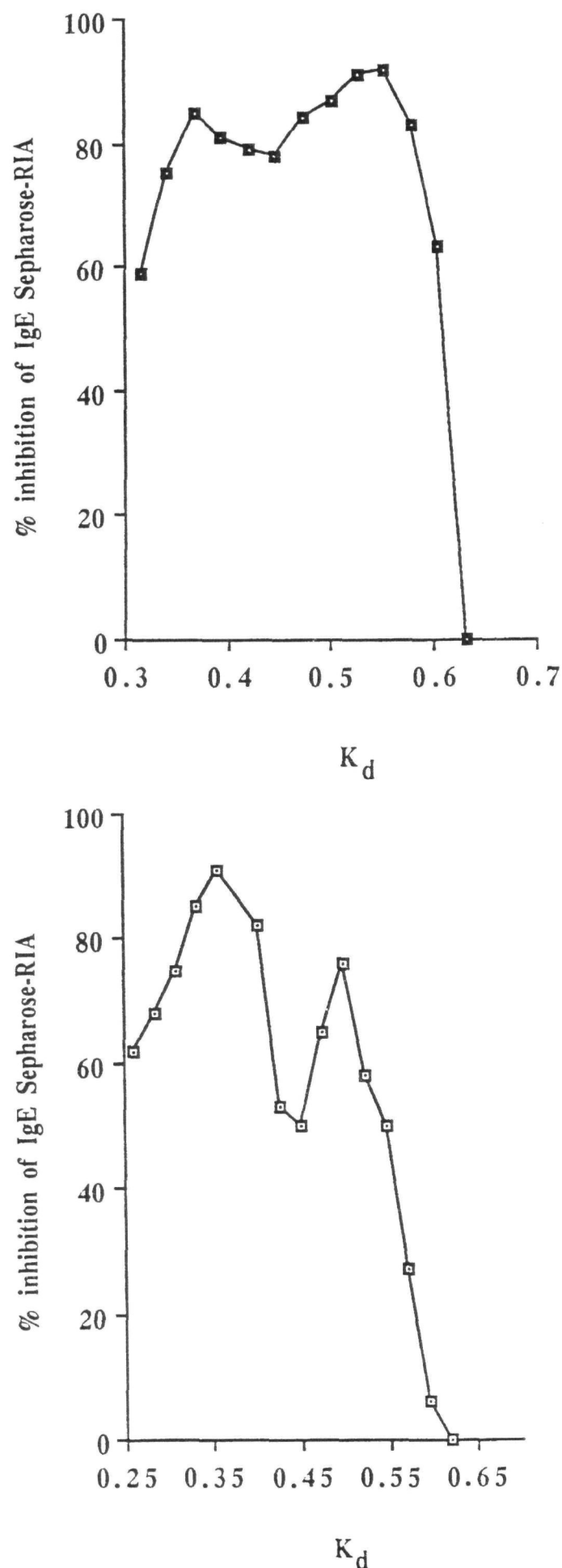


Fig. 3. Inhibition of surimi-IgE-RIA after incubation of patient serum 5 with 50 µl of surimi extract (bottom) and codfish extract (top) eluted from Sephacryl S200 gel filtration. Each fraction is numbered as value of distribution coefficient of gel filtration, as calculated by formula $K_d = \frac{V_i - V_o}{V_t - V_o}$ where V_o = void volume, V_t = volume of fraction tested. K_d refers to logarithm of mol. mass of protein. Two peaks named I and II were identified as containing fish allergen.

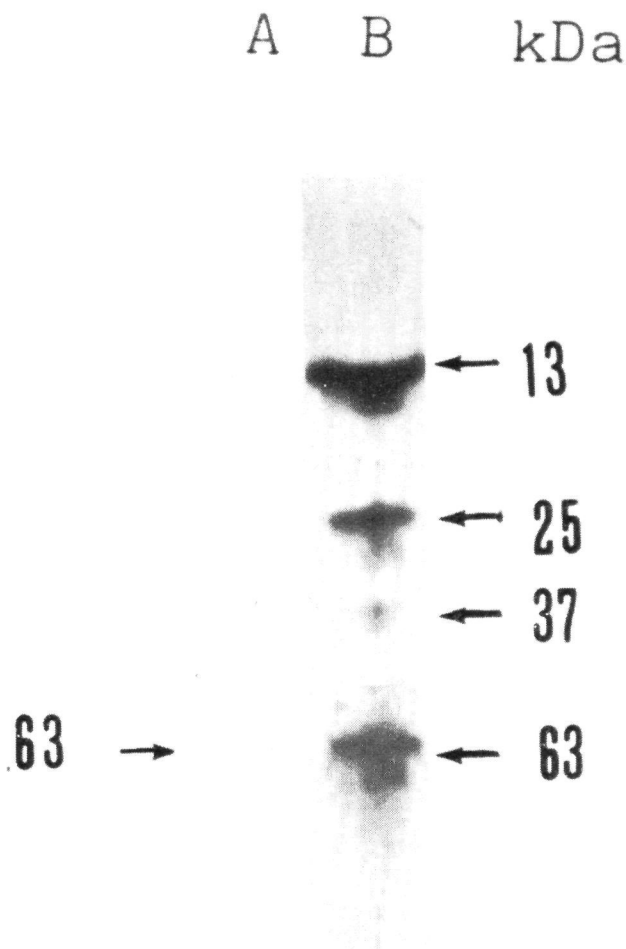


Fig. 4. SDS-PAGE of peak I from gel filtration of either surimi (lane A) or codfish extract (lane B). Migration was from bottom to top. Respective mol. mass of protein bands are indicated in margin.

fact, ingestion of surimi may also induce type I reaction in patients with allergy to egg because commercial surimi contains egg white (14).

In the present study, by electrophoresis of the codfish gel filtration eluate, we identified a 13-kDa protein which could correspond to the *Gad c I* allergen described by Elsayed & Apold (8). However, this protein was not found in the surimi eluate. The protein which was found in SDS-PAGE of both gel filtration eluates from surimi and codfish had a mol. mass of 63 kDa (Fig. 4). This protein band may represent an additional allergenic protein, which is different from *Gad c I*, and which is present in the myofibrillar proteins of codfish. Another hypothesis is that *Gad c I* derives from this 63-kDa protein and could be produced by proteolysis or reductive denaturation.

In conclusion, surimi contains an allergenic protein which has a mol. mass estimated at 63 kDa.

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