

Identification and characterization of a 30 kd major allergen from *Parapenaeus fissurus*

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The allergenic components of the shrimp (Parapenaeus fissurus) were identified by immunoblotting, with sera from 10 allergic patients. Six components, ranging in molecular weight from about 86 to 39 kd, showed IgE-binding activity and were identified as allergens of the shrimp. The component with a molecular weight of about 39 kd showed the highest frequency of IgE binding (70%) and was considered to be one of its major allergens. Two monoclonal antibodies against this 39 kd component were generated, and their antigenic cross-reactivity with five different kinds of seafood, shrimp, crab, cuttlefish, oyster, and pomfret was analyzed. Monoclonal antibody 1-6-10B reacted with the 39 kd component from shrimp only, but monoclonal antibody 2-7-1E also reacted with the 39 kd component from crab. By extraction with 0.5% sodium dodecylsulfate and ethanol precipitation, a highly purified shrimp 39 kd component was obtained. In two-dimensional gel electrophoresis six isoforms of this purified 39 kd component, with isoelectric point values from 5.1 to 5.6, were identified. No marked difference was observed when the amino acid composition of this purified 39 kd allergen was compared with those of serum albumin from different animals. They all contain a high proportion of acidic amino acids. There was also a 62% to 83% sequence homology among three different pairs of peptide fragments of purified 39 kd components of shrimp and crab. In conclusion, a 39 kd major allergen from the shrimp has been identified and characterized in the present study. According to the suggestions of the International Union of Immunological Societies, this allergen is designated as Par f I. (J ALLERGY CLIN IMMUNOL 1993;92:837-45.)

Key words: Shrimp, allergen, monoclonal antibody

Seafood has been recognized as a major causative agent of food allergy.¹ However, with the exception of the M protein from codfish,²⁻⁴ little is known about other allergens of seafood origin.

Components with molecular weight (MW) of about 8.2, 20, 26, 34, 36, and 38 kd have been reported as allergens from shrimp.⁵⁻⁸ However, further characterization of these components should be provided according to the suggestions by the International Union of Immunological Societies. In the present study the allergenic components of the most common shrimp from Taiwan

Abbreviations used

CBB-R250:	Coomassie Brilliant Blue R250
MoAb:	Monoclonal antibody
MW:	Molecular weight
pI:	Isoelectric point
PTH:	Phenylthiohydantoin
SDS:	Sodium dodecylsulfate
SDS-PAGE:	Sodium dodecylsulfate-polyacrylamide gel electrophoresis

(*Parapenaeus fissurus*) were recognized by patients' sera and analyzed by immunoblotting. Monoclonal antibodies (MoAbs) against a major allergen of the shrimp were also generated for allergen identification and were used in studies on the cross-reactivity among different kinds of seafood. The molecular charge of this major allergen was analyzed by two-dimensional gel electrophoresis. After purification by extraction with 0.5% sodium dodecylsulfate (SDS) and ethanol precipitation, we also determined the amino acid composition and sequence of this allergen.

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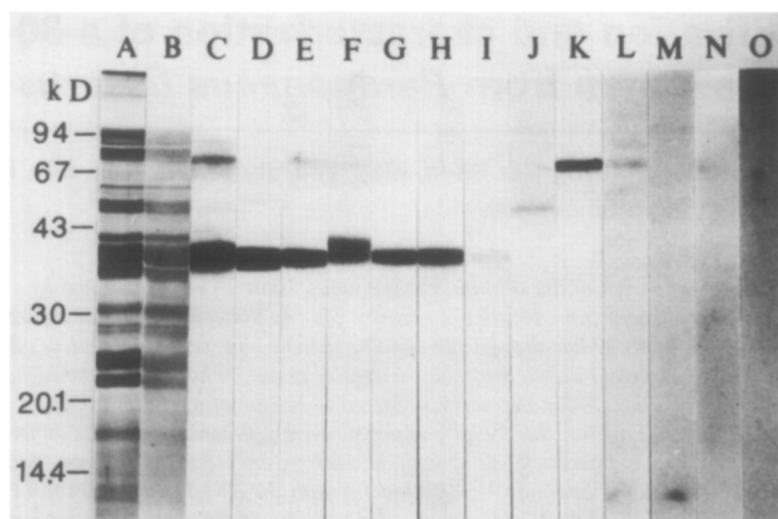


FIG. 1. Identification of IgE-binding components of the crude extract from shrimp (*Parapenaeus fissurus*) by SDS-PAGE and immunoblotting. Lanes A and B are CBB-R250-stained protein profiles of the crude extract of *Parapenaeus fissurus* in SDS-polyacrylamide gel and on nitrocellulose membrane after electrophoretic transfer, respectively. Lanes C to L are blots incubated with different serum samples from patients allergic to shrimp. Lanes M to O are blots incubated with cord sera. The MW standards used were phosphorylase b, 94 kd; bovine serum albumin, 67 kd; ovalbumin, 43 kd; carbonic anhydrase, 30 kd; soybean trypsin inhibitor, 20.1 kd; and α -lactalbumin, 14.4 kd.

TABLE I. The frequency of specific IgE binding of sera from patients to shrimp (*Parapenaeus fissurus*) allergens identified by immunoblot analysis

Shrimp allergen fraction	MW (kd)	Frequency* (%)
1	86	20
2	74	40
3	50	10
4	47	20
5	41	10
6	39	70

*The frequency of IgE binding was calculated as follows: No. of serum samples that demonstrated IgE-binding to each component $\div$ no. of serum samples with positive immunoblotting activity $\times$ 100.

METHODS

Crude extracts from seafood

Shrimp (*Parapenaeus fissurus*), crab (*Portunus (portunus) trituberculatus*), cuttlefish (*Sepia (sepia) esculenta*), oyster (*Crassostrea gigas*), and pomfret (*Pampus argenteus*) used in the present study were purchased from a local market in Taipei, Taiwan. The edible portions were removed and homogenized in phosphate-buffered saline (containing 137 mmol/L of NaCl, 2.7 mmol/L of KCl, 8 mmol/L of Na_2HPO_4 , 14.7 mmol/L of KH_2PO_4 , pH 7.4; 1:100 wt/vol) for 3 minutes with

TABLE II. Characteristics of mouse monoclonal antibodies against the 39 kd major allergen of the shrimp (*Parapenaeus fissurus*)

Antibody	Immunoglobulin isotype	Activity to <i>P. fissurus</i> *
1-6-10B	IgG ₁ , Kappa	2.584
2-7-1E	IgG ₁ , Kappa	1.645

*The activity was determined with undiluted hybridoma culture supernatants by ELISA. Absorbance of the supernatant at 415 nm was recorded.

the use of a blender. They were subsequently extracted with the same buffered saline solution at 4° C for 16 hours. After centrifugation, ammonium sulfate was added slowly to the supernatant to produce 100% saturation. The paste-like precipitate collected after centrifugation (12,000 g at 4° C for 30 minutes) was dissolved in and dialyzed against deionized water for 24 hours at 4° C. The dialysate was sterilized by syringe filtration (0.45 μm , Minisart, Gottingen, Germany) and stored in 2 ml aliquots at 4° C after lyophilization. The protein content of the extract was determined with the dye-binding method (Bio-Rad Laboratories, Richmond, Calif.).

Serum samples

Serum samples used in this study were collected from patients who attended the Dermatology Clinic at

Tri-Service General Hospital, Taipei. They ranged in age from 14 to 33 years old, and they had a history of allergy to shrimp and a positive immediate-skin test result to the crude shrimp extract. We analyzed the presence of specific IgE antibodies to *Parapenaeus fissurus* in these serum samples by radioimmunoassay. Samples were added individually to the wells of a flexible polyvinyl microtiter plate (Costar Corp., Cambridge, Mass.), which was coated with the crude extract of *Parapenaeus fissurus*. The radioactivity of each sample was then counted in a gamma counter (LKB, Turku, Finland). After incubation with iodine 125-labeled monoclonal anti-human IgE antibodies.⁹ Cord serum samples were used as the negative control.

Sodium dodecylsulfate–polyacrylamide gel electrophoresis, two-dimensional gel electrophoresis, and immunoblotting

Sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE) or two-dimensional gel electrophoresis was carried out as described previously.^{10, 11} The components of crude extracts from seafood that were separated by gel electrophoresis and recognized by IgE antibodies in sera or MoAbs were analyzed by immunoblotting.^{10, 11} After electrotransferring and blocking, the blot was incubated with diluted serum samples or with hybridoma culture supernatants at room temperature for 16 hours or 1 hour, respectively. After washing, the blot was incubated with ¹²⁵I-labeled monoclonal anti-human IgE antibodies or horseradish peroxidase–conjugated goat anti-mouse IgG (H + L) (Bio-Rad Laboratories) antibodies at room temperature for 1 hour each. Antigen-antibody binding was identified by autoradiography or by development in horseradish peroxidase substrate (0.06% 4-chloro-1-naphthol and 0.01% H₂O₂). Cord serum samples or culture supernatants of SP2 cells were used as the negative control.

Generation of MoAbs

MoAbs against the major allergen of *Parapenaeus fissurus* were generated as described previously.¹² Spleen cells obtained from BALB/c mice immunized with the gel containing the major allergen were fused with murine plasmacytoma SP2 cells in the presence of polyethylene glycol (MW = 1500 d; Merck, Hohenbrunn, Germany). Antibody-producing hybrid cells were screened by ELISA and SDS-PAGE immunoblotting with the crude extract of *Parapenaeus fissurus*. Hybridomas producing MoAbs against this major allergen were subcloned at least twice by limiting dilutions and then further expanded. The isotype of the MoAbs was determined by ELISA with class-specific anti-sera (Zymed Laboratories, San Francisco, Calif.).

Purification of protein components from SDS-polyacrylamide gels

Components in the crude extracts from seafood were purified by extraction from the protein-containing SDS-

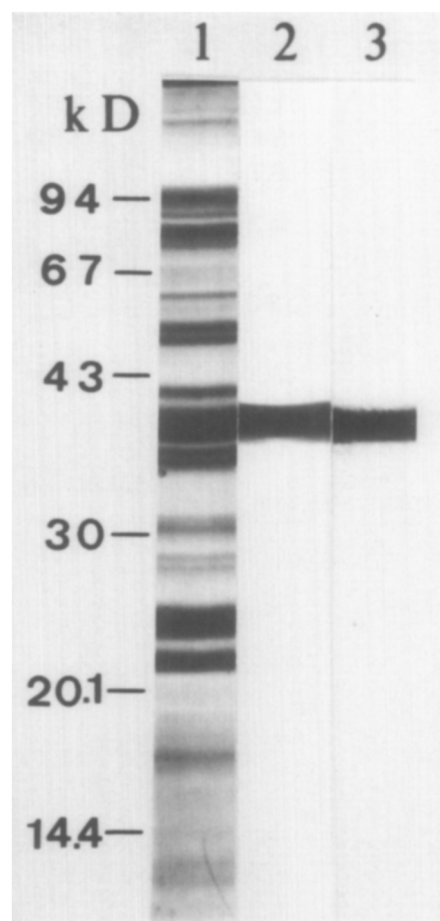


FIG. 2. Immunoblot profiles of the crude extract from shrimp (*P. fissurus*) separated by SDS-PAGE and identified with MoAbs. Lane 1 shows the CBB-R250–stained protein profile of the shrimp extract. Lanes 2 and 3 show immunoblot profiles identified with MoAbs 1-6-10B and 2-7-1E, respectively.

polyacrylamide gel slips stained with Coomassie Brilliant Blue (CBB) R250 (Imperial Chemical Industries, Macclesfield, Cheshire, England) with 0.5% SDS solution for 6 days. After centrifugation, the supernatant was collected and lyophilized. The lyophilized powder was washed at 4° C with 40% ethanol three times to remove staining dyes and SDS, and the precipitated proteins were then dissolved in deionized water. The protein-containing solution was lyophilized again and stored at 4° C.

Analysis of amino acid composition

The amino acid composition of purified seafood components was determined by a high-performance amino acid analyzer (model 6300, Beckman Instruments, Inc., Fullerton, Calif.).

Analysis of amino acid sequence

The purified protein components were digested individually in 100 mmol/L of Na₂CO₃, pH 9.5, with the

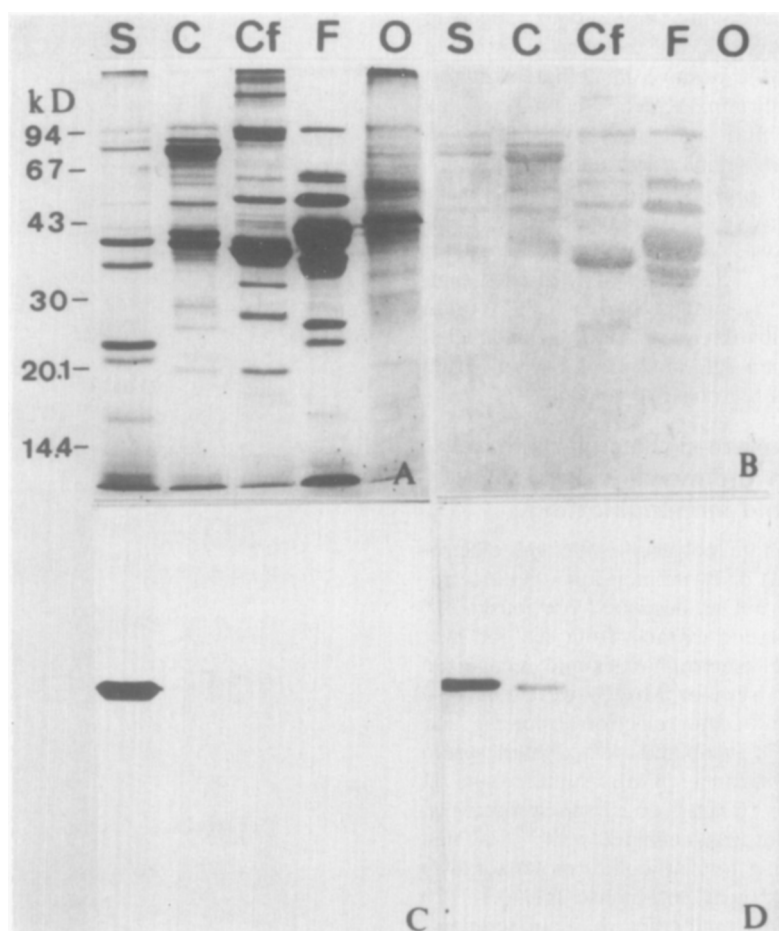


FIG. 3. Immunoblot profiles of crude extracts from five different kinds of seafood separated by SDS-PAGE and identified with MoAbs. **Panels A and B** show the protein profiles of the seafood extracts in SDS-polyacrylamide gel and on nitrocellulose membrane, respectively. *S*, *C*, *Cf*, *O*, and *F* represent the crude extracts from the shrimp (*Parapenaeus fissurus*), crab (*Portunus portunus trituberculatus*), cuttlefish (*Sepia (sepia) esculenta*), oyster (*Crassostrea gigas*), and pomfret (*Pampus argenteus*), respectively. **Panels C and D** show the immunoblot profiles of different kinds of seafood as shown in **panels A and B** identified with MoAbs 1-6-10B and 2-7-1E, respectively.

endopeptidase Lys-C (Wako, Osaka, Japan) at 30° C for 18 hours, with an enzyme-to-substrate ratio of 1:50.^{13, 14} After enzyme digestion, peptide fragments were purified by reverse-phase high-performance liquid chromatography with a Pierce Aquapore RP-300 column (4.6 mm internal diameter × 22 cm length, Pierce Chemical Co., Rockford, Ill.). Peptides were eluted in gradients with 0.1% trifluoroacetic acid in water (buffer A) and 0.08% trifluoroacetic acid in acetonitrile (buffer B). The gradient program used was: elution 0% buffer B in 2 minutes, 0% to 75% buffer B in 50 minutes, 75% to 100% buffer B in 5 minutes, 100% buffer B in 3 minutes, 100% to 0% buffer B in 2 minutes, and 5-minute reequilibration with a solvent flow rate of 1 ml/min. The effluents were monitored at both 220 and 280 nm.

Automated cycles of Edman degradation were carried out with a gas-liquid phase sequencer (ABI

model 470A, Applied Biosystems, Inc., Foster City, Calif.) and an on-line phenylthiohydantoin (PTH)-amino acid analyzer (ABI model 120A, Applied Biosystems, Inc.). The sequencing program was carried out with a subroutine consisting of a 5-minute trifluoroacetic acid treatment followed by 180-second butyl chloride and 240-second ethyl acetate washes.¹⁵ The resulting PTH-amino acids were analyzed by an ABI PTH-C18 reverse-phase cartridge column (2.1 mm internal diameter × 22 cm length; Applied Biosystems, Inc.). The PTH-amino acids eluted were quantitated by a data module (model 740, Waters Association, Milford, Mass.).

RESULTS

Protein components of the crude extract from shrimp were separated by SDS-PAGE, and the

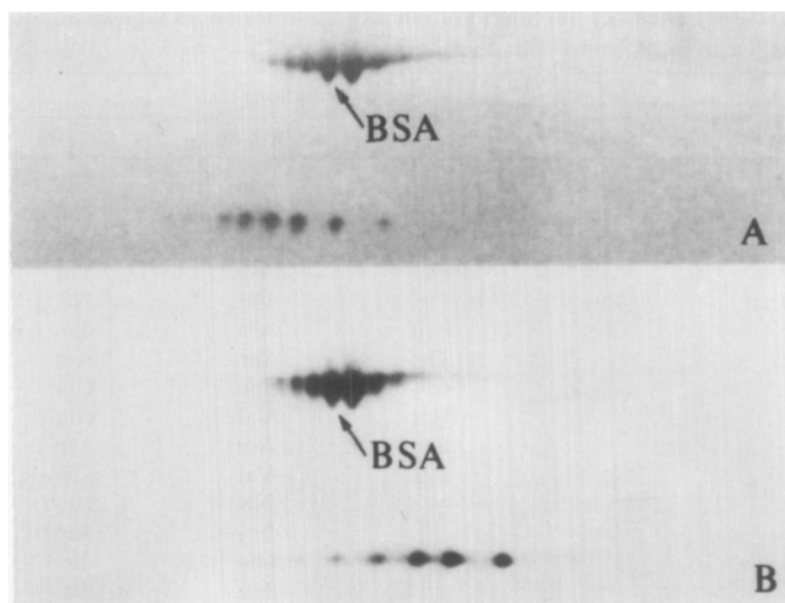


FIG. 4. Silver-stained two-dimensional gel electrophoresis protein profiles of the purified 39 kd components from the shrimp (*Parapeneaus fissurus*) (A) and crab (*P. (portunus) trituberculatus*) (B). An arrow indicates bovine serum albumin (BSA), which was used as an internal standard.

allergenic components were identified with IgE antibodies in immunoblotting. The CBB-R250-stained protein profile of the crude extract is illustrated in Fig. 1, A. Although at least 19 different components with MWs ranging from more than 100 to about 10 kd were identified, the predominant components had MWs of about 23, 24, 39, and 88 kd. For immunoblot analysis, serum samples from 10 patients who were allergic to shrimp were used (Fig. 1, C-L). The specific IgE counts of the 10 serum samples as analyzed by radioimmunoassay were all more than two times the value obtained with cord sera (data not presented). In general, sera with higher specific IgE counts showed higher intensity of IgE-staining bands in immunoblotting. A total of six allergenic components, ranging in MW from 86 to 39 kd, were recognized by IgE antibodies (Fig. 1 and Table I). The component with a MW of about 39 kd showed the highest frequency of IgE binding, and was recognized by 70% of the sera tested (Table I). Thus it was considered to be a major allergen of shrimp. The component with a MW of about 74 kd, which demonstrated a frequency of IgE binding of 40%, was also considered a significant allergen. Other components showed IgE-binding frequencies equal to or less than 20% (Table I). For negative control, IgE-binding activity was not observed when cord serum samples were used (Fig. 1, strips M-O).

TABLE III. Amino acid compositions of the purified 39 kd components of the shrimp (*Parapeneaus fissurus*) and crab (*Portunus (portunus) trituberculatus*)

Amino acid	No. of residues	
	Shrimp 39 kd	Crab 39 kd
Asp	39	36
Thr	18	19
Ser	16	17
Glu	47	52
Pro	13	18
Gly	33	27
Ala	25	21
Val	21	23
Met	8	8
Ile	16	14
Leu	32	31
Tyr	11	14
Phe	20	18
His	10	10
Lys	29	29
Arg	15	16
Cys	0	0
Trp	ND	ND
Total	353	353

ND, Not determined.

TABLE IV. Amino acid sequences of peptide fragments of the purified 39 kd component of the shrimp (*Parapenaeus fissurus*) digested with endopeptidase Lys-C

Cycle	Peptide fractions					
	Sh39-4	Sh39-5	Sh39-7	Sh39-10	Sh39-13	Sh39-14
1	Glu	Leu	Gly	Asp	Gly	Thr
2	Val	Glu	Thr	Phe	Phe	Ser
3	Phe	Ala	Tyr	Gly	Leu	Leu
4	Asp	Gly	Phe	Asp	Val	Gly
5	Lys	Phe	Pro	Val	NS	Ala
6		Lys	Leu	Asn	Ile	NS
7			Thr	Ser	Asn	Leu
8			Gly	Phe	Glu	Leu
9			Met	Val	Glu	Asp
10			Ser	Asn	Asp	Val
11			Lys	Val	His	Ile
12				NS	Leu	Gln
13				Pro	Arg	Ser
14				Glu	Ile	Gly
15				NS	Ile	Val
16				Gln	Ser	NS
17				Phe	NS	NS
18				Val	Gln	Leu
19				Ile	NS	Asp
20				Ser	Gly	Ser
21						Gly
22						NS
23						Asp
24						Ile
25						Tyr
26						Ala

NS, No sequence could be assigned.

After fusion of the immunized spleen cells and plasmacytoma SP2 cells, two MoAbs, 1-6-10B and 2-7-1E, against this 39 kd major allergen of shrimp were obtained. The ELISA and immunoblotting activities of both MoAbs to the crude extract from shrimp are shown in Table II and Fig. 2, respectively. The isotype of both MoAbs was IgG₁, and both had kappa light chains (Table II).

For the analysis of cross-reactivity among different kinds of seafood, both MoAbs against the 39 kd major allergen of shrimp were used. Fig. 3 shows that among the five different kinds of seafood tested, MoAb 1-6-10B reacted only with the 39 kd component from shrimp. MoAb 2-7-1E showed immunoblotting activity to both 39 kd components of shrimp and crab. For negative control, no color reaction was observed when the culture supernatant of SP2 cells was used (data not presented).

The 39 kd components from shrimp and crab were purified by extraction of protein-containing gels with 0.5% SDS and ethanol precipitation. Both purified 39 kd components showed six different isoforms, as analyzed by two-dimensional

gel electrophoresis (Fig. 4). The isoelectric point (pI) values were from 5.1 to 5.6 for the purified 39 kd component from shrimp and from 5.2 to 5.9 for the purified 39 kd component from crab (Fig. 4). All of the six different isoforms of the purified 39 kd component from shrimp showed immunoblotting activity to MoAbs 1-6-10B and 2-7-1E, whereas the purified 39 kd component from crab showed immunoblotting activity to MoAb 2-7-1E only (data not presented).

Table III shows that both purified 39 kd components of shrimp and crab had similar amino acid composition. They contained 353 amino acids and were rich in glutamic acid (glutamine), aspartic acid (asparagine), leucine, lysine, and glycine.

After purification, the peptides of the 39 kd components from shrimp and crab were digested by endopeptidase Lys-C, and each of the six fractions was selected for amino acid sequence analysis. The results are shown in Tables IV and V. A sequence homology of about 62% to 83% among three different pairs of peptides from the shrimp and crab was observed (Table VI).

TABLE V. Amino acid sequences of peptide fragments of the purified 39 kd component of the crab (*Portunus (portunus) trituberculatus*) digested with endopeptidase Lys-C

Cycle	Peptide fractions					
	Cr39-2a	Cr39-2b	Cr39-5	Cr39-9a	Cr39-9b	Cr39-19
1	Tyr	Val	Leu	Asp	Gly	Thr
2	Leu	Asn	Glu	Phe	Arg/Asn	NS
3	Tyr	Glu	Glu	Gly	Met	Leu
4	Lys	Leu	Gly	Asp	Asp	Gly
5	Ser	Tyr	Phe	Val	Leu	Ala
6	Lys	Gln	Lys	Asn	Thr	Thr
7		Lys		Gln	Glu	Leu
8				Phe	Tyr	Leu
9				Val	Gln	Asp
10				Asn	Ala	Val
11				Val		Ile
12				Asp		Gln
13				Pro		Ser
14				Asp		Gly
15				Gly		Val
16				Lys		Glu
17				Lys		Asp
18						Leu
19						NS
20						NS
21						Gly

NS, No sequence could be assigned.

DISCUSSION

We identified six components from shrimp (*Parapenaeus fissurus*), ranging in MW from 39 to 86 kd, with immunoblotting activity to IgE antibodies in serum of patients allergic to shrimp. The component with a MW of about 39 kd that showed the highest frequency of IgE binding (70%) was considered a major allergen of shrimp. After purification, molecular charge, amino acid composition, and sequence of this 39 kd component were analyzed. According to the suggestions of the International Union of Immunological Societies, this 39 kd major allergen from shrimp with pI values from 5.1 to 5.6 is designated as *Par f* I.

On the basis of gel-filtration analysis, Hoffman et al.⁵ reported a heat-stable major allergen from shrimp, antigen-II, with a MW of about 38 kd and pI values from 5.4 to 5.8 (Table VII). However, with the use of gel filtration and ion-exchange chromatography, Nagpal et al.⁶ reported a 34 kd heat-stable major allergen from the shrimp (*Penaeus indicus*), Sa-II (Table VII). In the present study a similar heat-stable major allergen from the shrimp (*Parapenaeus fissurus*), *Par f* I, with a MW of about 39 kd and pI values from 5.1 to 5.6 has also been identified by SDS-PAGE immunoblotting with sera from patients allergic to shrimp (Table VII).

Results from analysis of amino acid composition showed that all three shrimp allergens mentioned above were rich in glutamic acid (glutamine), aspartic acid (asparagine), leucine, and lysine. Such an amino acid composition pattern is not remarkable, compared with those of serum albumins from different animals.¹⁶ Furthermore, it was found that most of the native proteins characterized are acidic proteins.¹⁷ Because glutamic acid and aspartic acid belong to the group of major acidic amino acids,¹⁸ the high content of glutamic acid and aspartic acid may not be a unique characteristic of allergens from shrimp but more likely a common property of acidic proteins.

Results mentioned above suggest that *Par f* I, antigen II, and even Sa-II may represent the same or a very similar major allergen of the shrimp. The observed differences may be due to use of different species of shrimp and/or different analytic methods. Although at present it is not clear whether all of these allergens belong to the same molecule, the amino acid sequence of *Par f* I discovered in the present study and the two MoAbs, 1-6-10B and 2-7-1E, generated against *Par f* I should provide more information for comparison.

In the present study both MoAbs against the 39 kd major allergen of the shrimp were also used for

TABLE VI. Homology of amino acid sequence between peptide fragments of the 39 kd components of the shrimp and crab

Cycle	Sh39-5	Cr39-5	Sh39-10	Cr39-9a	Sh39-14	Cr39-19
1	(Leu)	(Leu)	(Asp)	(Asp)	(Thr)	(Thr)
2	(Glu)	(Glu)	(Phe)	(Phe)	Ser	NS
3	Ala	Glu	(Gly)	(Gly)	(Leu)	(Leu)
4	(Gly)	(Gly)	(Asp)	(Asp)	(Gly)	(Gly)
5	(Phe)	(Phe)	(Val)	(Val)	(Ala)	(Ala)
6	(Lys)	(Lys)	(Asn)	(Asn)	NS	Thr
7			Ser	Gln	(Leu)	(Leu)
8			(Phe)	(Phe)	(Leu)	(Leu)
9			(Val)	(Val)	(Asp)	(Asp)
10			(Asn)	(Asn)	(Val)	(Val)
11			(Val)	(Val)	(Ile)	(Ile)
12			NS	Asp	(Gln)	(Gln)
13			(Pro)	(Pro)	(Ser)	(Ser)
14			Glu	Asp	(Gly)	(Gly)
15			NS	Gly	(Val)	(Val)
16			Gln	Lys	NS	Glu
17			Phe	Lys	NS	Asp
18			Val		(Leu)	(Leu)
19			Ile		Asp	NS
20			Ser		Ser	NS
21					(Gly)	(Gly)
22					NS	
23					Asp	
24					Ile	
25					Tyr	
26					Ala	

Parentheses indicate the same amino acid residues appeared in pairs of peptide fragments of the 39 kd components of the shrimp and crab.

NS, No sequence could be assigned.

TABLE VII. Characteristics of the major allergens of the shrimp reported by different authors

Allergen	Shrimp	MW (kd)	pI	Amino acid composition	Amino acid sequence	Authors
<i>Par f</i> I	<i>Parapenaeus fissurus</i>	39	5.1 to 5.6	+	+	Lin et al.*
Antigen II	—	38	5.4 to 5.8	+	—	Hoffman et al. ⁵
Sa-II	<i>Penaeus indicus</i>	34	—	+	—	Nagpal et al. ⁶

Plus signs indicate that tabulated characteristics have been evaluated, and minus signs indicate that they have not.

*Present study.

analysis of cross-reactivity among antigens of five different kinds of seafood. MoAb 1-6-10B had a strong reaction to the 39 kd major allergen from shrimp only. However, MoAb 2-7-1E reacted to both 39 kd components of the shrimp and crab. This suggests that different epitopes on this 39 kd major allergen from shrimp may be recognized by these two MoAbs and that homology of amino acid sequences of the 39 kd components from shrimp and crab may exist.

Furthermore, results from amino acid sequence analysis showed about 62% to 83% sequence

homology among three different pairs of peptide fragments of the 39 kd components of the shrimp and crab. Analyzed by SDS-PAGE immunoblotting, the 39 kd component of crab also reacted strongly with IgE antibodies in serum samples from two patients allergic to crab (data not presented).

In conclusion, the present study identified *Par f* I as a major allergen of the shrimp (*Parapenaeus fissurus*). Cross-reactivity between *Par f* I and the 39 kd allergen of crab was also disclosed by use of MoAbs and by amino acid sequencing.

REFERENCES

1. Atkins FM, Steinberg SS, Metcalfe DD. Evaluation of immediate adverse reactions to foods in adult patients. II. A detailed analysis of reaction patterns during oral food challenge. *J ALLERGY CLIN IMMUNOL* 1985;75:356-63.
2. Metcalfe DD. Food allergens. *Clin Rev Allergy* 1985;3:331-49.
3. Lemanske RF Jr, Taylor SL. Standardized extracts, foods. *Clin Rev Allergy* 1987;5:23-36.
4. Marsh DG, Goodfriend L, King TP, Lowenstein H, Platts-Mills TAE. Allergen nomenclature. *J ALLERGY CLIN IMMUNOL* 1987;80:639-45.
5. Hoffman DR, Day ED, Miller JS. The major heat stable allergen of shrimp. *Ann Allergy* 1981;47:17-22.
6. Nagpal S, Rajappa L, Metcalfe DD, Subba Rao PV. Isolation and characterization of heat-stable allergen from shrimp (*Penaeus indicus*). *J ALLERGY CLIN IMMUNOL* 1989;83:26-36.
7. Morgan JE, Daul CB, Lehrer SB, English M. Characterization of important shrimp allergens by immunoblot analysis [Abstract]. *J ALLERGY CLIN IMMUNOL* 1990;85:170.
8. Daul CB, Slattery M, Morgan JE, Lehrer SB. Isolation and characterization of an important 36 kD shrimp allergen [Abstract]. *J ALLERGY CLIN IMMUNOL* 1991;87:192.
9. Chang ZN, Chang LD, Wang MC, et al. Monoclonal antibodies specific for human IgE and their clinical applications. *Proc Natl Sci Counc Repub China B* 1988;12:140-5.
10. Shen HD, Wang SR, Tang RB, Chang ZN, Su SN, Han SH. Identification of allergens and antigens of Bermuda-grass (*Cynodon dactylon*) pollen by immunoblot analysis. *Clin Allergy* 1988;18:401-9.
11. Shen HD, Choo KB, Lin WL, Lin RY, Han SH. An improved scheme for the identification of antigens recognized by specific antibodies in two-dimensional gel electrophoresis and immunoblotting. *Electrophoresis* 1990;11:878-82.
12. Shen HD, Chang LY, Su SN, Han SH. Characteristics of five monoclonal antibodies to major allergens of the short ragweed pollen. *Int Arch Allergy Appl Immun* 1988;85:167-73.
13. Masaki T, Fujihashi T, Nakamura K, Soejima M. Studies on a new proteolytic enzyme from *Achromobacter lyticus* M497-1. I. Purification and some enzymatic properties. *Biochim Biophys Acta* 1981;24:44-50.
14. Masaki T, Fujihashi T, Nakamura K, Soejima M. Studies on a new proteolytic enzyme from *Achromobacter lyticus* M497-1. II. Specificity and inhibition studies of *Achromobacter* protease I. *Biochim Biophys Acta* 1981;24:51-5.
15. Hsieh JC, Lin FP, Tam MF. Electrophoretic transfer onto glass-fiber filter from an analytical isoelectrofocusing gel: a preparative method for isolating proteins for N-terminal microsequencing. *Anal Biochem* 1988;170:1-8.
16. Peters T. Serum albumin. *Adv Clin Chem* 1970;13:37-111.
17. Ginazza E, Righetti PG. Size and charge distribution of macromolecules. *J Chromatogr* 1980;193:1-8.
18. Hardy PM. The protein amino acids. In: Barrete GC, ed. *Chemistry and biochemistry of the amino acids*. London: Chapman and Hall Co., 1985:6-24.