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Immunological characteristics of monoclonal antibodies against shellfish major allergen tropomyosin

Ying Lu [a], Toshiaki Ohshima [a], Hideki Ushio [a,*], Yuki Hamada [b], Kazuo Shiomi [a]

[a] Department of Food Science and Technology, Tokyo University of Marine Science and Technology, 5-7 Konan 4, Minato, Tokyo 108-8477, Japan
[b] Faculty of Fisheries, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki-shi 852-8521, Japan

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Abstract

Two types of murine monoclonal antibodies (MAbs) against American lobster (*Homarus americanus*) were generated and characterized. Three purified MAbs were characterized to be specific to the shellfish major allergen tropomyosin. MAbs 5G5E1 and 1A3A7 were reactive to tropomyosin from crustacean species only, whereas MAb 2A7H6 was reactive to both crustacean and mollusk tropomyosins. None of the antibodies reacted to vertebrate tropomyosins. Competitive ELISA indicated that the antigenic epitopes recognized by the two types of MAbs were different from each other. In addition, competitive immunoblot results showed that the binding of shellfish-allergic patient IgEs to lobster tropomyosin was inhibited by the MAb 2A7H6 only. This finding suggests that the antigenic epitope for the 2A7H6 antibody might be similar or close to the allergenic epitope shared by crustaceans and mollusks. Consequently, the MAbs recognizing the different common antigenic epiotopes obtained in the present study would not only facilitate the allergen characterization of shellfish, but may also be useful for the development of specific and sensitive immunoassays for allergen quantification or epitope mapping.

Keywords: Monoclonal antibody; Tropomyosin; Allergen; Shellfish allergy

1. Introduction

Shellfish is a broad term for all aquatic animals that have a shell or shell-like exoskeleton. In general, shellfish are separated into two basic categories, namely crustaceans and mollusks. Shellfish is not only an important food source for human, but also a frequent cause of adverse food hypersensitivity, which is commonly synonymous with IgE-mediated immediate-type I allergy. It has been reported that hypersensitivity reactions to crustaceans and mollusks could cause various clinical symptoms such as urticaria, asthma, diarrhea and others (Daul, Morgan, & Lehrer, 1993).

Food allergens are food component(s) that induce the production of IgE antibodies and cross-bind to these antibodies to cause chemical mediator releases from mast cells and basophils, resulting in immediate and hypersensitive reactions (Taylor & Lehrer, 1996). Tropomyosin was first identified as a major allergen in shrimp (Daul, Slattery, Reese, & Lehrer, 1994; Shanti, Martin, Nagpal, Metcalfe, & Subba Rao, 1993). Studies on shellfish allergy indicated that tropomyosin is a major cross-reactive allergen among crustaceans and mollusks (Halmepuro, Salvaggio, & Lehrer, 1987; Lehrer & McCants, 1987; Leung et al., 1996). In recent years, tropomyosin has been demonstrated to be a major allergen for various shellfish, including shrimp (Daul et al., 1994; Leung et al., 1994; Shanti et al., 1993), lobster (Leung et al., 1998), crab (Leung et al., 1998), squid (Miyazawa et al., 1996), oyster (Ishikawa, Shimakura, Nagashima, & Shiomi, 1997; Leung & Chu, 2001), and octopus (Ishikawa, Suzuki, Ishida, Nagashima, & Shiomi, 2001) and is believed to be a pan-allergen of invertebrates including arthropoda and mollusks (Reese, Ayuso, & Lehrer, 1999).

Since immunoassays based on the MAbs specific to allergens are helpful for allergen characterization and

* Corresponding author. Tel.: +81 3 5463 0615; fax: +81 3 5463 0627.
E-mail address: hushio@s.kaiyodai.ac.jp (H. Ushio).

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quantification (Chapman, Heymann, Wilkins, Brown, & Platts-Mills, 1987; Jeoung et al., 1997; Portnoy, Brothers, Pacheco, Landuyt, & Barnes, 1998), we have established murine MAbs against Japanese abalone (*Haliotis discus*) major allergen tropomyosin in a previous study (Lu, Ohshima, Ushio, & Shiomi, 2004). However, it seems to be difficult to select the MAbs recognizing the common antigenic structures shared by mollusk species. In the present study, murine MAbs against American lobster were generated in order to establish monoclonal antibodies against shellfish common allergen tropomyosin. The immunological characteristics of the MAbs obtained were also investigated and discussed.

2. Materials and methods

2.1. Materials

Live specimens of American lobster (*Homarus americanus*), Japanese common squid (*Todarodes pacificus*), whelk (*Babylonia japonica*) and baby clam (*Tapes japonica*) were obtained from a local seafood market in Tokyo, Japan. Frozen specimens of Japanese abalone (*Haliotis discus*) and king crab (*Paralithodes camtschatica*) were purchased from a local supermarket. Fresh specimens of Japanese oyster (*Crassostrea gigas*), octopus (*Octopus vulgaris*), common scallop (*Patinopecten yessoensis*) adductor muscle and pallium, snow crab (*Chionoecetes opilio*), kegani-crab (*Erimacrus isembeckii*), black tiger prawn (*Penaeus monodon*), Chinese prawn (*Penaeus chinensis*), common carp (*Cyprinus carpio*), sardine (*Sardinops melanosticta*), chicken (*Gallus gallus*) leg muscle and chicken gizzard were purchased from local suppliers. Patient sera were obtained from three individuals with histories of allergic reactions to both crustaceans and mollusks.

2.2. Immunogen preparation

Acetone powders of myofibrillar proteins from lobster and squid were prepared according to the method reported by Greaser and Gergely (1971) with slight modifications. The fresh abdominal muscle of lobster and the mantle muscle of squid were individually minced and extracted in a buffer A (1:5, w/v) containing 20 mM KCl, 1 mM $KHCO_3$, 0.1 mM $CaCl_2$ and 0.1 mM dithiothreitol (DTT) for 10 min. All extracts were separately filtered twice with 4 layers of gauze and then rinsed three times with the buffer A. After centrifugation at 15,220*g* for 20 min, the obtained precipitates were washed four times with acetone. Acetone was then evaporated at room temperature for 1–2 h. To obtain the tropomyosin-rich extracts, the acetone powders were extracted overnight in buffer B (1:15, w/v) containing 1 M KCl, 25 mM Tris, pH 8.0, 1 mM $KHCO_3$, 0.1 mM $CaCl_2$ and 0.1 mM DTT. After centrifugation at 20,000*g* for 25 min, the supernatants were fractionated with 35% and 55% ammonium sulphate solutions. After determining the protein concentration by Bradford (1976) method, the resulting 35–55% ammonium sulphate fractions of lobster and squid were aliquoted and stored at −20 °C.

American lobster *H. americanus* tail muscle tropomyosin was reported to have an apparent molecular weight of about 38 kDa (Miegel, Kobayashi, & Maeda, 1992). Therefore, the lobster tropomyosin proteins nearby 38 kDa were purified by preparative sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE), followed by electroelution as previously described (Lu et al., 2004). After quantifying the protein concentration, the purified tropomyosins were stored at −85 °C.

2.3. Preparation of samples from different materials

The boiled samples of different crustaceans (shrimp and crab) and mollusks (octopus, oyster, whelk, abalone, clam and scallop) and myofibrillar protein samples of vertebrates (carp, sardine, frog, chicken and chicken gizzard) were individually prepared according to the procedures described in a previous report (Lu et al., 2004).

2.4. Production of MAbs

The 35–55% ammonium sulphate fraction of lobster was used as the immunogen for MAb preparation. Two BALB/cA mice (female, 6 weeks) were immunized intraperitoneally with 100 μL of emulsions prepared by mixing the lobster immunogen (200 μg/mL) with complete Freund's adjuvant (1:1, v/v) at the 0 day and with incomplete Freund's adjuvant at the 14th day, respectively. After testing the antibody titer of immunized mouse antiserum by enzyme linked immunosorbent assay (ELISA) as described below, 20 μg of lobster immunogen in Dulbecco's phosphate buffered saline (PBS) containing 8.00 g/L NaCl, 1.15 g/L Na_2HPO_4, 0.20 g/L KCl, 0.20 g/L NaH_2PO_4 (pH 7.4) was intravenously injected at the 21st day.

Three days after the intravenous injection, the splenic lymphocytes from the immunized mouse were fused with mouse myeloma cells (P3-X63-Ag8.U1) by the method of Köhler and Milstein (1975). Hybridomas were selected by incubation in a GIT medium (Wako Pure Chemical Industries, Ltd., Osaka, Japan) containing 10% BM-Condimed H1 (Roche Diagnostics, Tokyo, Japan) and 1% hypoxanthine, aminopterin and thymidine supplement (HAT, Roche Diagnostics). Following screening by ELISA, the antibody-producing hybridomas were cloned three times by a limiting dilution method (Köhler & Milstein, 1975). Finally, the selected MAbs were purified by a Protein-G column (HiTrap™ Protein G HP, Amersham Bioscience, Tokyo, Japan), and the isotypes of the obtained MAbs were determined using a mouse monoclonal antibody isotyping kit (Roche Diagnostics, Tokyo, Japan).

2.5. Biotinylation of MAbs

Biotinylation of MAbs was performed with an EZ-Link Sulfo-NHS-LC-Biotinylation kit (Pierce Biotechnology,

Inc., Rockford, IL, USA) according to the manufacturer's introduction. Unreacted biotin was removed by a D-Salt dextran desalting column packaged in the kit.

2.6. Enzyme linked immunosorbent assay

Enzyme linked immunosorbent assay (ELISA) was used to determine the antibody titers of the immunized animals and to screen the antibody-producing hybridomas. Lobster and squid tropomyosin-rich extracts were used as antigens for hybridoma screening. All procedures were performed at room temperature. In brief, 100 μL of antigen samples (5 μg/mL) were separately coated onto a flat-bottom microtiter plate (Costar®, Corning International, Tokyo, Japan) by incubation for 1 h. After washing four times with Tris buffered saline (pH 7.6, TBS) (Takara Bio Inc., Otsu, Shiga, Japan) containing 1.24 g/L Tris (hydroxymethyl) aminomethane, 6.27 g/L Tris (hydroxymethyl) aminomethane hydrochloride, and 8.77 g/L NaCl plus 0.5% Tween 20 (TBS-T), the wells were blocked with TBS containing 0.05% Tween 20 and 1% bovine serum albumin (BSA) for 1 h. Following the washing with TBS-T, the antiserum dilutions from the immunized animals or the culture supernatants (100 μL) were added and incubated for 1 h. Horseradish peroxidase-labeled goat anti-mouse IgM, IgG, IgA (HRP-GAM Igs, 1:10,000, v/v, Southern Biotechnology Associated, Inc., Birmingham, AL, USA) was used as a secondary antibody. The bound antibodies were detected by addition of 100 μL of freshly made substrate solution of *o*-phenylenediamine dihydrochloride (OPD, Sigma–Aldrich Japan, Tokyo, Japan). After the enzyme reaction was stopped with 3 mol/L sulphuric acid, the absorbance at 490 nm was measured on a densitometer (CS-9300 PC, Shimadzu Corp., Kyoto, Japan) equipped with a multi-well apparatus for 96 well plates.

Competitive ELISAs were used to investigate the relationship of the epitopes recognized by the obtained MAbs as follows. First, purified lobster tropomyosin (1 μg/mL) was coated on a 96-well microtiter plate overnight at 4 °C. Following the blocking, the immobilized tropomyosin was pre-incubated with serial dilutions of the MAbs ranging from 20 μg/mL to 10 ng/mL, and successively incubated with biotinylated MAbs (0.5 μg/mL) for 1.5 h at 37 °C. Finally, the specific binding was detected by the reaction between streptavidin–biotin-peroxidase (1:100, v/v, Wako Pure Chemical Industries, Osaka, Japan) and OPD substrate.

2.7. SDS–PAGE and immunoblot assay

The obtained murine MAbs against lobster tropomyosins were characterized by immunoblot assays. The different invertebrate and vertebrate samples were used as antigens for the following immunoblot assays. SDS–PAGE was performed according to the Laemmli (1970), and all procedures were performed at room temperature. In brief, proteins from different antigens were applied to 12.5% gels and stained with Coomassie brilliant blue (CBB) R-250. The separated proteins were electrophoretically transferred to polyvinylidene difluoride (PVDF, Millipore Corp., Tokyo, Japan) membranes according to the method of Towbin, Staehelin, and Gordon (1979). Optimum dilutions of purified MAbs were used as primary antibodies. The specific reactions were detected by a combination of HRP-GAM Igs (1:10,000, v/v) or rat HRP-labeled anti-human IgE secondary antibody (HRP-RAH IgE, 1:8000, v/v, Southern Biotechnology Associated, Birmingham, AL, USA) and the SuperSignal® West Pico chemiluminescent substrate (Pierce Biotechnology).

2.8. IgE binding inhibition by MAbs

In order to investigate whether the epitopes recognized by the obtained MAbs were similar to those recognized by the serum IgEs of shellfish-sensitive patients, an inhibition assay for IgE binding ability was carried out as follows. One microgram of lobster antigen was blotted onto a nitrocellulose membrane (Trans-Blot®, Bio-Rad Laboratories, Hercules, CA, USA) by aspirating through a Dot Blotter (Sanplatec Corp., Osaka, Japan). Following blocking, the immobilized antigen was pre-incubated with serial dilutions of the MAbs ranging from 0.01 ng/mL to 50 μg/mL for 90 min. After washing with TBS-T, the membrane was subsequently incubated with a dilution of patient serum (1:100, v/v) for 90 min. Finally, the bound antibodies were detected by HRP-RAH IgE secondary antibody (1:8000, v/v) and the SuperSignal® West Pico chemiluminescent substrate (Pierce Biotechnology, Rockford, IL, USA). The intensities of the chemiluminesence at every MAb concentration were analyzed by an ImageJ V. 1.31u developed at the US National Institute of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/.

3. Results and discussion

3.1. Production of monoclonal antibodies

For the hybridomas generated by lobster immunogen, from the total of 322 hybridomas, 223 clones producing antibodies with positive response to lobster antigen only, and 34 clones with reaction to both lobster and squid antigens were obtained. Ten hybridoma clones were selected for successive screening and cloning. Among the clones producing antibodies with reactivity to both lobster and squid, MAb 2A7H6 was purified by protein-G column and identified as IgG1 with κ light chains. In addition, MAbs 5G5E1 (IgG2b) and 1A3A7 (IgG1) with reactivity only to lobster were also purified.

3.2. Characterization of MAbs

Western blotting for lobster and squid myofibrillar proteins were carried out to evaluate the specific reactivity of the MAbs 2A7H6, 5G5E1 and 1A3A7. As shown in Fig. 1, all the MAbs (1A3A7, 5G5E1 and 2A7H6) showed reactivity

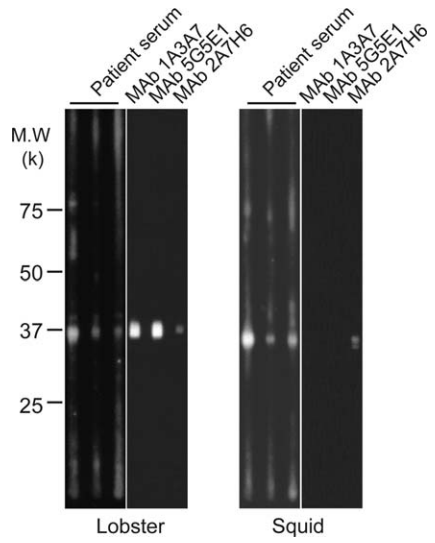

Fig. 1. Specific reactivity of the MAbs examined by Western blotting. Lobster *H. americanus* and squid *T. pacificus* antigens were applied to a 12.5% SDS–PAGE. Sera from three shellfish-sensitive patients (1:100, v/v) and purified MAbs 1A3A7, 5G5E1 and 2A7H6 (1 μg/mL individually) were used as primary antibodies. The bound antibody was detected by HRP-RAH IgE (1:3000, v/v) and HRP-GAM Igs (1:10,000, v/v) secondary antibodies in combination with chemiluminescent substrate.

to the lobster proteins with molecular weight nearly 37 kDa, and only the MAb 2A7H6 also reacted to the squid proteins with similar molecular weights. In addition, both the lobster and squid proteins detected by the MAbs were found to react with the sera IgEs from three crustacean allergic patients (Fig. 1). The MAb 2A7H6 was cross-reactive to proteins with molecular weights within 30–40 kDa from different boiled extracts from crustaceans and mollusks, while the MAbs 5G5E1 and 1A3A7 were reactive only to the crustaceans. However, none of the MAbs were reactive to the myofibrillar proteins of vertebrates including chicken, frog, carp, sardine, and chicken gizzard (Fig. 2).

Tropomyosin was reported to be a cross-reactive allergen in crustaceans and mollusks (Leung et al., 1996; Leung & Chu, 1998). A serial of heat-stable tropomyosins with a molecular weight ranging from 34 to 38 kDa have been identified to be major allergens of different crustacean and mollusk species (Daul et al., 1994; Ishikawa et al., 2001; Leung & Chu, 2001; Shanti et al., 1993). For the American lobster *H. americanus* and squid *T. pacificus*, muscle tropomyosin around 38 kDa was reported to be major allergens of these species (Miyazawa et al., 1996; Leung et al., 1998). The MAbs obtained in the present study showed reactivity not only to the allergenic proteins 37 kDa of lobster and squid, but also to the proteins around 37 kDa of other shellfish species. These results indicated that the MAbs were probably reactive to the common shellfish allergen tropomyosin.

Interestingly, the MAb 2A7H6 was not reactive to bivalves baby clam and common scallop even though it reacted to bivalve oyster, cephalopods (squid and octopus), gastropods (whelk and abalone) and crustaceans (lobster, shrimp and crab) (Fig. 2). This finding indicates that the 2A7H6 antibody would recognize one common epitope shared by crustaceans and in part by mollusk species. Among the mollusk species such as oyster, abalone and mussel, overall sequence homology for tropomyosins is only about 50–60%, and the IgE-binding epitopes of mollusk species also suggested to be polymorphic (Chu, Wong, & Leung, 2000). Our finding that the 2A7H6 antibody reacted partly to some mollusk species led us to consider that the antigenic epitope shared by baby clam and common scallop might be different from that shared by the other mollusk species. Also, it indirectly implied that the antigenic epitopes of the mollusk allergen tropomyosins might be polymorphic as well as the allergenic epitopes.

3.3. Relationship of the antigenic structures recognized by MAbs

In work reported previously, we established the MAbs specific to the Japanese abalone *H. discus* major allergen tropomyosin. Among the MAbs obtained, one MAb

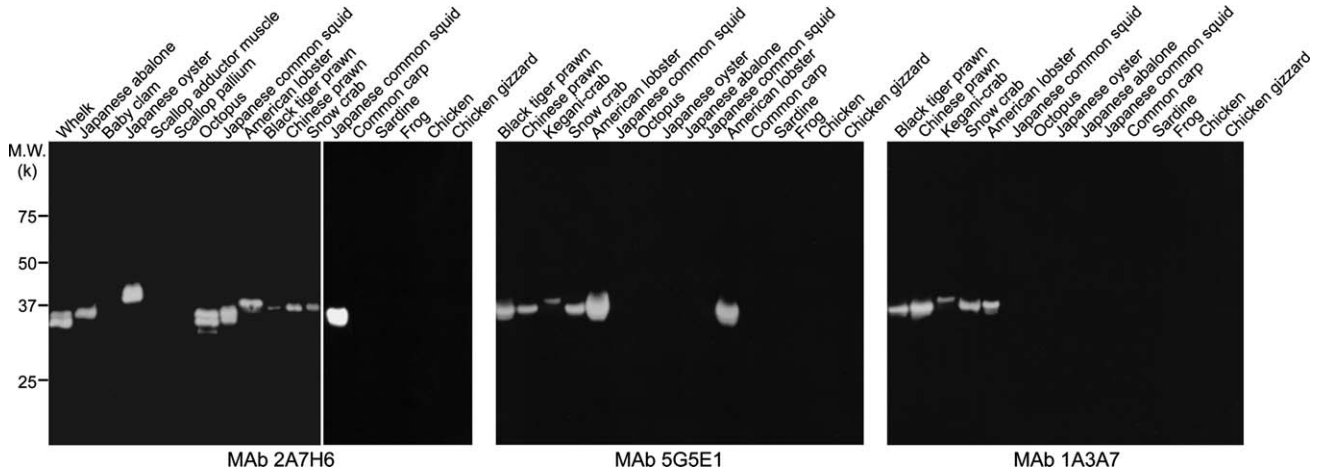

Fig. 2. Cross-reactivity of the MAbs to different crustaceans, mollusks and vertebrates. Purified MAbs 2A7H6 (4 μg/mL), 5G5E1 and 1A3A7 (2.5 μg/mL) were used as primary antibodies, and HRP-GAM Igs (1:10,000, v/v) was used as secondary antibody, respectively.

AE9F9 recognized the epitope shared by different crustaceans, abalone and vertebrate chicken was obtained (Lu et al., 2004). In the present study, the relationships of the antigenic structures recognized by the MAbs 5G5E1, 1A3A7, 2A7H6 and AE9F9 were individually investigated by competitive ELISAs. As shown in Fig. 3A, the biotinylated MAb 1A3A7 showed reduced binding to the lobster tropomyosin preincubated with the competitor MAb 5G5E1; as high as about 85% inhibition in binding was observed when preincubated with a concentration of 20 μg/mL MAb 5G5E1. However, the binding of the biotinylated MAbs 5G5E1 and 1A3A7 to the tropomyosin

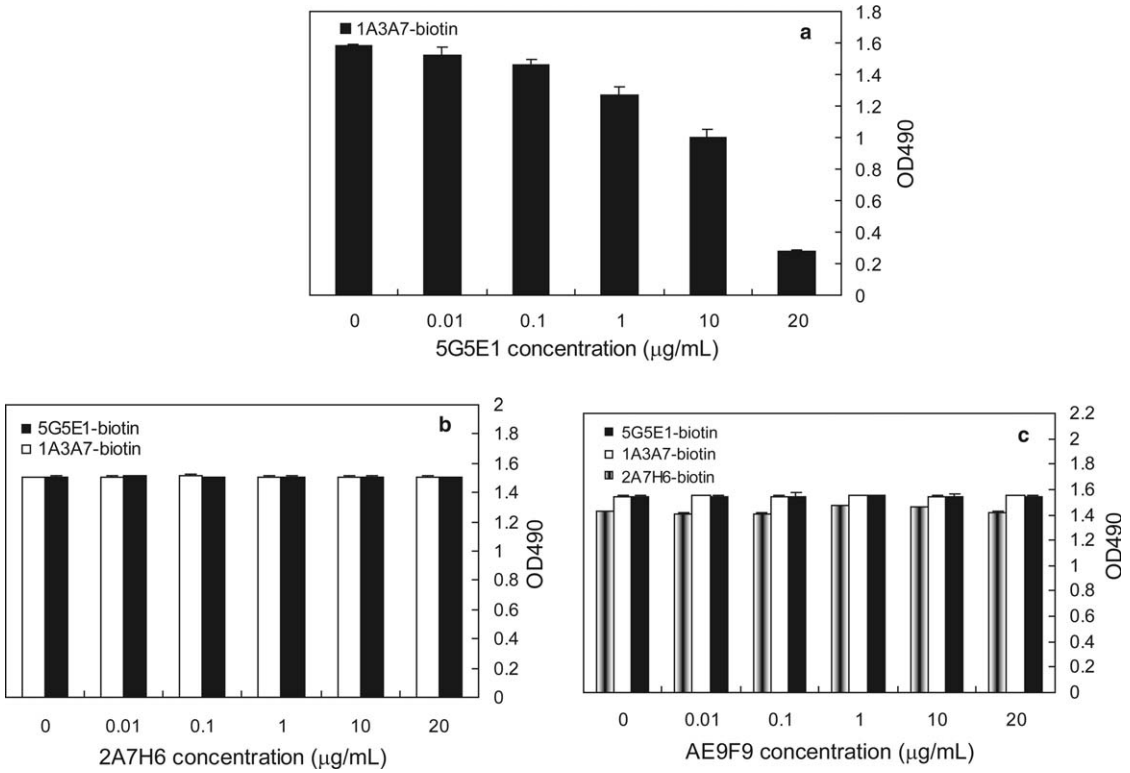

Fig. 3. Relationship of the antigenic epitopes for MAbs investigated by inhibition ELISA. Following pre-incubation with various dilutions of competitor MAbs, the immobilized lobster tropomyosin (0.1 μg/well) was then incubated with the biotinylated MAbs (0.5 μg/mL individually). Immunodetection was performed by the reaction between streptavidin–biotin-peroxidase (1:100, v/v) and OPD substrate.

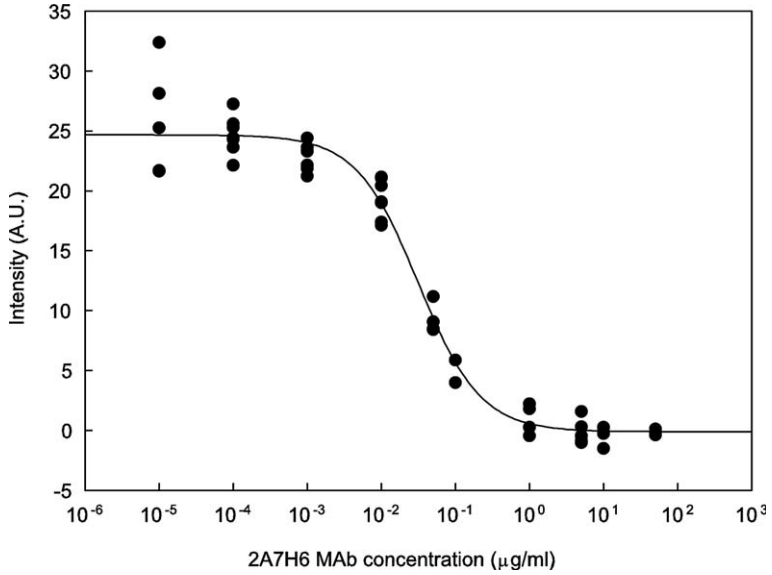

Fig. 4. Inhibitory effect of the binding between IgEs and the immobilized lobster tropomyosin by MAb 2A7H6. Membrane was pre-incubated with serial dilutions of the competitor MAb 2A7H6, and subsequently incubated with patient serum (1:100, v/v). Immunodetection was performed by incubation with HRP-GAH IgE (1:8000, v/v) secondary antibody in combination with chemiluminescent substrate.

was little reduced by the competitor 2A7H6 (Fig. 3B). Similarly, no significant reduction in the binding of the biotinylated MAbs 5G5E1, 1A3A7 and 2A7H6 to the tropomyosin was observed when the MAb AE9F9 was used as the competitor (Fig. 3C). These results indicated that the MAbs 5G5E1 and 1A3A7 would probably possess similar or close antigenic epitopes different from those for the MAbs 2A7H6 and AE9F9, also suggesting that the MAbs might be applicable for epitope mapping.

On the basis of competitive immunoblot result, it was found that the 2A7H6 antibody showed an inhibitory effect on the reactivity between patient serum IgEs and the lobster tropomyosin with an ID_{50} of around 50 ng/mL (Fig. 4), while the other MAbs failed to inhibit the IgE binding to the tropomyosin (data not shown). Previous studies suggested that a common allergenic structure would be present among the tropomyosins of crustaceans and mollusks (Lehrer & McCants, 1987; Leung et al., 1996; Reese et al., 1999). The inhibition data naturally led us to associate the common allergenic epitope with this common antigenic one. Thus, we assume that the antigenic epitope for the 2A7H6 antibody might be close or similar to the common allergenic epitope(s) of the patient IgE antibody. Further studies are necessary to identify the epitopes recognized by the obtained MAbs and to investigate their relationship with the IgE-binding epitopes of the shellfish common allergen tropomyosin.

4. Conclusions

In the present study, we established two types of MAbs that were specific to the shellfish major allergen tropomyosin. The finding that these two types of MAbs recognized different antigenic epitopes suggests that the MAbs might contribute to epitope mapping. In addition, the MAbs 5G5E1 and 1A3A7 would be suitable for application to the characterization of crustacean allergens because they were able to recognize the common allergen tropomyosin of crustacean species. The present study also showed that the antigenic epitope for MAb 2A7H6 might be close or similar to the allergenic epitopes shared by crustaceans and mollusks. From available limitations for patient sera, the MAb 2A7H6, which can be generated illimitably, would be useful for allergen characterization and detection. In conclusion, we think that the MAbs recognizing different epitopes will facilitate not only the allergen characterization, but also specific and sensitive immunoassay development.

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References

Bradford, M. M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*, 248–254.

Chapman, M. D., Heymann, P. W., Wilkins, S. R., Brown, M. J., & Platts-Mills, T. A. E. (1987). Monoclonal immunoassays for major dust mite (Dermatophagoides) allergens, Der p 1 and Der f 1, and quantitative analysis of the allergen content of mite and house dust extracts. *The Journal of Allergy and Clinical Immunology, 80*, 184–194.

Chu, K. H., Wong, S. H., & Leung, P. S. C. (2000). Tropomyosin is the major mollusk allergen: reverse transcriptase polymerase chain reaction, expression and IgE reactivity. *Marine Biotechnology, 2*, 499–509.

Daul, C. B., Morgan, J. E., & Lehrer, S. B. (1993). Hypersensitivity reactions to crustacea and mollusks. *Clinical Reviews in Allergy, 11*, 201–222.

Daul, C. B., Slattery, M., Reese, G., & Lehrer, S. B. (1994). Identification of the major brown shrimp (*Penaeus aztecus*) as the muscle protein tropomyosin. *International Archives of Allergy and Immunology, 105*, 49–55.

Greaser, M. L., & Gergely, J. (1971). Reconstitution of troponin activity from three protein components. *The Journal of Biological Chemistry, 246*, 4226–4233.

Halmepuro, L., Salvaggio, J. E., & Lehrer, S. B. (1987). Crawfish and lobster allergens: identification and structural similarities with other crustacea. *International Archives of Allergy and Immunology, 84*, 165–172.

Ishikawa, M., Shimakura, K., Nagashima, Y., & Shiomi, K. (1997). Isolation and properties of allergenic proteins in the oyster *Crassostrea gigas*. *Fisheries Science, 63*, 610–614.

Ishikawa, M., Suzuki, F., Ishida, M., Nagashima, Y., & Shiomi, K. (2001). Identification of tropomyosins as a major allergen in the octopus *Octopus vulgaris* and elucidation of its IgE-binding epitopes. *Fisheries Science, 67*, 934–942.

Jeoung, B. J., Reese, G., Hauck, P., Oliver, J. B., Daul, C. B., & Lehrer, S. B. (1997). Quantification of the major brown shrimp allergen Pen a 1 (tropomyosin) by a monoclonal antibody-based sandwich ELISA. *The Journal of Allergy and Clinical Immunology, 100*, 229–234.

Köhler, G., & Milstein, C. (1975). Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature, 256*, 495–497.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, 227*, 680–685.

Lehrer, S. B., & McCants, M. L. (1987). Reactivity of IgE antibodies with crustacea and oyster allergens: evidence for common antigenic structures. *The Journal of Allergy and Clinical Immunology, 80*, 133–139.

Leung, P. S. C., Chen, Y. C., Gershwin, M. E., Wong, S. H., Kwan, H. S., & Chu, K. H. (1998). Identification and molecular characterization of *Charybdis feriatus* tropomyosin, the major crab allergen. *The Journal of Allergy and Clinical Immunology, 102*, 847–852.

Leung, P. S. C., Chen, Y. C., Mykles, D. L., Chow, W. K., Li, C. P., & Chu, K. H. (1998). Molecular identification of the lobster muscle protein tropomyosin as a seafood allergen. *Molecular Marine Biology and Biotechnology, 7*, 12–20.

Leung, P. S. C., Chow, W. K., Ansari, A., Bandea, C. I., Kwan, H. S., Nagy, S. M., et al. (1994). Cloning, expression, and primary structure of *Metapenaeus ensis* tropomyosin, the major heat-stable shrimp allergen. *The Journal of Allergy and Clinical Immunology, 94*, 882–890.

Leung, P. S. C., Chow, W. K., Duffey, S., Kwan, H. S., Gershwin, M. E., & Chu, K. H. (1996). IgE reactivity against a cross-reactive allergen in crustacea and mollusca: evidence for tropomyosin as the common allergen. *The Journal of Allergy and Clinical Immunology, 98*, 954–961.

Leung, P. S. C., & Chu, K. H. (1998). Molecular and immunological characterization of shellfish allergens. *Frontiers in Bioscience, 3*, 306–312.

Leung, P. S. C., & Chu, K. H. (2001). cDNA cloning and molecular identification of the major oyster allergen from the Pacific oyster *Crassostrea gigas*. *Clinical and Experimental Allergy, 31*, 1287–1294.

Lu, Y., Ohshima, T., Ushio, H., & Shiomi, K. (2004). Preparation and characterization of monoclonal antibody against abalone allergen tropomyosin. *Hybridoma and Hybridomics, 23*, 357–361.

Miegel, A., Kobayashi, T., & Maeda, Y. (1992). Isolation, purification and partial characterization of tropomyosin and troponin subunits from the lobster tail muscle. *Journal of Muscle Research and Cell Motility, 13*, 608–618.

Miyazawa, H., Fukamachi, H., Inagaki, Y., Reese, G., Daul, C. B., Lehrer, S. B., et al. (1996). Identification of the first major allergen of a squid (*Todarodes pacificus*). *The Journal of Allergy and Clinical Immunology, 98*, 948–953.

Portnoy, J., Brothers, D., Pacheco, F., Landuyt, J., & Barnes, C. (1998). Monoclonal antibody-based assay for Alt a 1, a major *Alternaria* allergen. *Annals of Allergy, Asthma and Immunology, 81*, 59–64.

Reese, G., Ayuso, R., & Lehrer, S. B. (1999). Tropomyosin: an invertebrate pan-allergen. *International Archives of Allergy and Immunology, 119*, 247–258.

Shanti, K. N., Martin, B. M., Nagpal, S., Metcalfe, D. D., & Subba Rao, P. V. (1993). Identification of tropomyosin as the major shrimp allergen and characterization of its IgE-binding epitopes. *Journal of Immunology, 151*, 5354–5363.

Taylor, S. L., & Lehrer, S. B. (1996). Principles and characteristics of food allergens. *Critical Reviews in Food Science and Nutrition, 36*, S91–S118.

Towbin, H., Staehelin, T., & Gordon, J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proceedings of the National Academy of Sciences of the United States of America, 76*, 4350–4354.