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Effect of Maillard Reaction on Allergenicity of Scallop Tropomyosin

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Scallop tropomyosin (TM), the major allergen of shellfish, was prepared from adductor muscles and reacted with four reducing sugars to investigate the effect of the Maillard reaction on the allergenicity of TM. The IgE-binding ability of TM increased significantly with the progress of the reaction with glucose, ribose, and maltose, but not with maltotriose. The allergenicity was enhanced at the early stage of the Maillard reaction, and the trend of the effect depended on the type of reducing sugar used. 2,4,6-Trinitrobenzenesulfonic acid treatment of the lysine residues in TM showed that the protein surface charge resulting from the Maillard reaction had no effect on the enhancement of the allergenicity. Thus, the change in the allergenicity would be closely related to the structural change caused by the Maillard reaction.

KEYWORDS: Scallop; tropomyosin; marine invertebrate; seafood allergy; allergenicity; Maillard reaction

INTRODUCTION

Currently, food allergies are a serious social problem in Japan. According to a recent survey (1996−1999) by the Ministry of Health, Labor, and Welfare, 7% of Japanese people have some kind of food allergy, and seafoods occupy 50% of allergens in the adults. Compared with agricultural and livestock products, many more marine bioresources (about 500 species) are consumed in Japan, and new kinds of hypersensitivity to seafood have been reported in the past decade. Although much research has been carried out to investigate seafood as a cause of food allergy (*1*−*4*) and the cross-reactivity among marine bioresources as food materials (*5*−*8*), far more information is required to reduce the damage of seafood allergies.

Most cases of food allergy are mediated by the IgE antibody. The binding of the IgE antibody to the epitope site of a specific protein (allergen) is the trigger of a series of allergenic responses. In food processing, proteins in food materials undergo structural changes, such as aggregation, polymerization, degradation, and random unfolding (*9*−*12*). These structural changes could affect the conformation of the vicinity of epitope sites in food allergens. Therefore, it is necessary to examine the effect of food processing on the specific IgE-binding ability of allergens. Food proteins often interact with other food components during processing and cooking. From the viewpoint of food chemistry, the Maillard reaction, which is a complex series of nonenzymatic reactions between the ϵ-amino group in proteins and the carbonyl group in reducing sugars, is one of the most important reactions because of its significant effect on food quality, including its flavor, color, texture, and nutritional value. Some researchers have reported that the Maillard reaction influences the allergenicity of food proteins and that the effect varies depending on the combination of proteins and reducing sugars. For example, the allergenicity of a major allergen in cherry decreased as a result of the Maillard reaction with glucose and ribose (*13*). In contrast, the allergenicity of β-lactoglobulin was enhanced by the Maillard reaction with lactose (*14*).

In seafood processing, the Maillard reaction occurs as fish, shellfish, shrimp, and squid are being dried. Dried scallop, one of the most important products in the seafood industry of northern Japan, has a high market value because it is widely used as an essential seasoning in Japanese, Chinese, and French dishes. The scallop adductor turns brown as a result of the Maillard reaction during the drying process, and this clear shiny brown color is an indicator of good quality in dried scallops. Scallop hypersensitivity is a frequent type of seafood allergy in Japan, and the allergen has been identified as TM (*15*). Because TM is a lysine-rich protein (lysine is 12% of the total amino acid of scallop TM; GenBank), it reacts easily with reducing sugars during the drying process. However, there is no information about the allergenicity of TM reacted with reducing sugars.

The objective of this study is to investigate the effect of the Maillard reaction on the allergenicity of scallop TM. Four reducing sugars, each with a different reactivity, were reacted

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Table 1. Clinical History of Patients' Sera Including Specific IgE Antibody against Scallop Tropomyosin

serum	age (years)	sex	hypersensitivity reaction	CAP-RAST class[a]	diluted ratio of serum: competitive ELISA	diluted ratio of serum: dot blotting
1	2	female	Ur and BA	4	1:300	1:500
2	3	female	Ur and BA	6	1:300	1:500
3	3	male	Ur and BA	3	1:100	1:200
4	2	male	Ur and BA	2	1:100	1:200
5	4	male	UR, BA, and OAS	3	1:25	1:20
6	3	male	BA and AD	2	1:20	1:20
7	2	male	Ur, BA, and OAS	3	1:10	
8	7	female	AD and OAS	2	1:20	1:20
9	2	male	Ur, Ba, and AD	4	1:100	1:200
10	5	male	Ur, BA, and Ana	3	1:20	1:20
11	1	male	AD and AB	3	1:100	1:200

[a] CAP-RAST is a diagnostic test to detect specific IgE antibody against allergen. Measured value is categorized as class 0–6.

with TM under a drying condition, and the change in the specific IgE-binding ability of TM was investigated using immunochemical procedures. In addition, the relationship between the allergenicity of TM and the structural change that occurs as a result of the Maillard reaction is discussed.

MATERIALS AND METHODS

Materials. Scallop (*Patinopecten yessoensis*) was purchased from a local fish market and frozen at −25 °C until use. BSA (fraction V) was obtained from Merck Co. Ltd. (Darmstadt, Germany). A calibration kit of glycosylated human serum was purchased from Japan Roche Diagnostic Inc. (Tokyo, Japan). All other chemicals (reagent grade) were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and Kanto Chemical Co., Inc. (Tokyo, Japan).

Preparation of Scallop TM. Scallop TM was prepared according to the method of Ojima et al. (*16*) with a slight modification. The sliced scallop striated adductor muscles (100 g) were washed and homogenized using a homogenizer (model AM-6, Nissei Co., Ltd., Tokyo, Japan) in 10 volumes of 20 mM Tris-HCl (pH 7.5) containing 0.1 M NaCl, 1 mM $MgCl_2$, 5 mM 2-ME, and 1 mM PMSF for 1 min at 15000 rpm. The homogenate was centrifuged at 5000*g* for 15 min, and the precipitate was resuspended in the same washing medium and further centrifuged three times. Finally, scallop myofibrils were collected as the precipitate and dissolved in 0.4 M LiCl containing 1 mM $NaHCO_3$ and 5 mM 2-ME, and the pH was adjusted to 5.0 with 0.1 M HCl. After gently stirring for 1 h and then centrifuging at 20000*g* for 30 min, the supernatant was collected, and the pH was adjusted to 7.5 with 0.1 M NH_4OH; the supernatant was then subjected to ammonium sulfate fractionation at 40−60% saturation. The precipitate was dissolved in the 0.4 M LiCl solution and centrifuged at 100000*g* for 2 h, and the supernatant was subjected to isoelectric precipitation at pH 4.65 with 0.05 M HCl. The precipitate was dissolved in 1 mM $NaHCO_3$ containing 5 mM 2-ME and dialyzed against the 0.4 M LiCl solution and also against 4 M urea containing 10 mM KCl, 5 mM 2-ME, and 10 mM Tris- HCl (pH 7.5). The crude TM obtained was further purified by anion exchange chromatography using a Toyopearl DEAE-650M (Tosoh Corp., Tokyo, Japan; column size: ∅ 1.6 × 40 cm). The crude TM (∼100 mg) was loaded onto the column equilibrated with the 4 M urea solution (containing 10 mM KCl, 5 mM 2-ME, and 10 mM Tris-HCl, pH 7.5) and eluted with a linear gradient of 10−300 mM KCl (pH 7.5) at a flow rate of 30 mL/h. The eluted TM fractions were collected and concentrated by ultrafiltration (Q0100/043E, exclusion limit molecular weight: 10000, Advantec, Tokyo, Japan) and dialyzed against 1 mM $NaHCO_3$ and 5 mM 2-ME. The protein concentration was determined using the protein assay rapid kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan) using BSA as a standard.

Human Serum. The sera of 11 subjects with the specific IgE antibody to scallop TM were selected. The clinical history of the patients is summarized in **Table 1**. Control sera were obtained from two subjects with no specific IgE antibody to scallop TM and two normal subjects who had shown no adverse reactions to any foodstuffs ingested. These sera were frozen at −25 °C until use. Scallop allergy was diagnosed on the basis of the detection of the TM-specific IgE antibody using the CAP-RAST (Pharmacia & Upjohn, Tokyo, Japan), positive skin test reactivity, and a positive response to the oral challenge test.

Maillard Reaction between TM and Reducing Sugars. TM dissolved in 1 mM $NaHCO_3$ and 5 mM 2-ME was mixed with various reducing sugars (glucose, ribose, maltose, and maltotriose) at a final concentration of 0.6 M, and the protein concentration was adjusted to 0.2 mg/mL. Each 0.7 mL of the mixture was placed into microtubes and frozen at −25 °C, immediately lyophilized (FDU-506; EYELA, Tokyo, Japan), and stored at −60 °C until use.

To further the Maillard reaction between TM and sugars, the lyophilized mixtures were incubated at 60 °C and relative humidity of 35% (RH 35%) in a temperature- and humidity-controlled cabinet (PR-1G; Tabai Espec Corp., Tokyo, Japan) for 48 h (glucose), 3 h (ribose), and 15 days (maltose and maltotriose). TMs reacted with sugars (TM-glucose, TM-ribose, TM-maltose, and TM-maltotriose) were redissolved in experimental buffers, and the protein concentrations were adjusted to 0.2 mg/mL by adding the buffers until the sample weight before lyophilization was achieved. The TMs reacted with reducing sugars (TM-sugars) thus obtained were subjected to the analysis described below.

Determination of Available Lysine and Ketoamine Contents. Available lysine and ketoamine assays were carried out to evaluate the progress of the Maillard reaction. The available lysine content was determined by the spectrophotometric analysis using *o*-phthalaldehyde and *N*-acetyl-L-cysteine (*17*). Before the analysis, TM reacted with sugars was dissolved in a 50 mM phosphate buffer (pH 9.5) containing 2% SDS at room temperature.

The ketoamine was assayed according to the method of Johnson et al. (*18*), and glycosylated human serum was used as a standard for determining the ketoamine content. Before the analysis of ketoamine, the protein solution was dialyzed against 0.1 M NaCl containing 20 mM Tris-HCl (pH 7.5) overnight at 4 °C to remove unreacted sugars.

Dot Blotting. A PVDF membrane (AE-6665; Atto Corp., Tokyo, Japan) was activated with methanol and soaked in PBS (0.14 M NaCl, 27 mM KCl, 86 mM Na_2HPO_4, and 14 mM KH_2PO_4, pH 7.4) for 1 h, and 20 μg of TMs dissolved in PBS was placed in each well of the immunoblot appliance (AE-6190; Atto Corp.) to adsorb onto the membrane. The membrane was blocked with 3% casein in TBS (0.15 M NaCl and 20 mM Tris-HCl, pH 7.5) at 25 °C for 1 h and subsequently incubated with the subjects' sera (diluted 1:20, 1:200, or 1:500 with 3% casein in TBS) overnight at 4 °C. The membrane was washed three times, for 10 min each time, with TBS containing 0.05% Tween 20 (TTBS) and an additional three times with TBS and incubated with peroxidase-conjugated rabbit anti-human IgE antibody (Dako Cytomation, Glostrup, Denmark; diluted 1:5000 with 3% casein in TBS) for 3 h at 25 °C. After the membrane was washed with TTBS and TBS three times, IgE reactivity was detected using an ECL mini-camera with ECL Western blotting detection reagents (Amersham, Buckinghamshire, U.K.) and Polaroid T-667 film (Polaroid Corp., Bedfordshire, U.K.). The photographs were reversed to obtain a white background using computer software (Photoshop ver. 4.0, Adobe, San Jose, CA).

Competitive ELISA. Patients' sera were incubated with various concentrations of TM-sugars, and the changes in the specific IgE-binding ability of the sera were investigated. A flat-bottom polystyrene microtiter plate (an ELISA plate with 96 wells; Iwaki, Tokyo, Japan) was coated with TM dissolved in PBS at 2.5 μg/mL overnight at 4 °C. After the plate had been washed four times with 350 μL of TPBS, the residual blocking site in the plate was coated with 150 μL of 1% BSA in PBS for 3 h at 37 °C and washed with PBS containing 0.05% Tween 20 (TPBS) four times. In another microtiter plate (an assay plate with 96 wells; Iwaki), 125 μL of each of the subjects' sera (diluted 1:10, 1:20, 1:25, 1:100, or 1:300 with 1% BSA in PBS) was mixed with equal volumes of TM-sugars as an inhibitor (0.002−200 μg/mL in PBS). After incubation at 37 °C for 2 h, each 70 μL of the solution was placed into the TM-coated ELISA plate described above.

The ELISA plate was incubated at 37 °C for 2 h and washed with TPBS four times. After incubation with 100 μL of peroxidase-

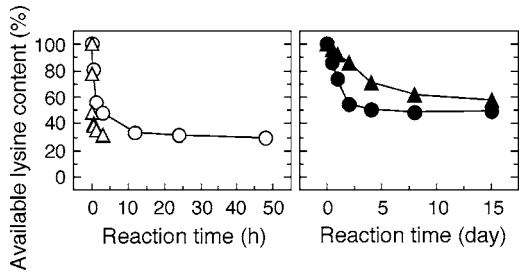

Figure 1. Changes in available lysine content in TM during reaction with glucose (○), ribose (△), maltose (●), and maltotriose (▲).

conjugated rabbit anti-human IgE antibody (diluted 1:2500 with 1% BSA in PBS; Dako Cytomation, Glostrup, Denmark) at 37 °C for 1.5 h, the plate was washed again and developed with 100 μL of a substrate solution (0.04% *o*-phenylenediamine dihydrochloride and 0.05% H_2O_2 in a 50 mM phosphate−citrate buffer, pH 5.0) at 25 °C for 20 min, and the absorbance was measured at 492 nm. Then, the enzyme reaction was stopped by the addition of 100 μL of 4 M H_2SO_4. The loss of the specific IgE-binding ability of the patients' sera resulting from the treatment with TM-sugars was represented by calculating the inhibition rate using the formula

$$\text{inhibition rate (\%)} = (X - Y) \div (X - Z) \times 100$$

where X is the absorbance of each patient's serum without TM-sugars and Y and Z are the absorbance of the patients' and control sera treated with various concentrations of TM-sugars, respectively.

In this study, the inhibitory concentration obtaining a 50% inhibition rate (defined as IC_{50}) was calculated from the inhibition curve in the competitive ELISA as an indicator of the allergenicity of TM (*19*, *20*).

TNBS Modification of Lysine Residues in TM. Various concentrations of TNBS (0.005−1.0 mM) were added to TM (final concentration = 0.4 mg/mL) dissolved in 0.2 M $NaHCO_3$ and incubated at 30 °C for 3 h to react with the lysine residues with TNBS. Before the reaction, TM was preincubated at 30 °C for 10 min. After being cooled in ice water, TM reacted with TNBS (TM-TNBS) was immediately dialyzed against PBS at 4 °C to remove unreacted TNBS.

Electrophoretic Analysis. SDS-PAGE was performed using a 12% acrylamide gel containing 0.1% SDS (*21*). Urea-PAGE was also carried out using a 4% acrylamide gel containing 8 M urea (*22*). After electrophoresis, the gels were stained with 0.25% Coomassie Brilliant Blue R-250 in 9% acetic acid and 45% methanol and destained with 30% methanol containing 7.5% acetic acid.

RESULTS AND DISCUSSION

Progress of Maillard Reaction between TM and Reducing Sugars. Figure 1 shows the change in the available lysine content in TM during the reaction with the reducing sugars. When lyophilized TM mixed with reducing sugars was incubated at 60 °C and RH 35%, the available lysine (0.110 g/g of protein) decreased markedly in all samples. Rapid loss of the available lysine was observed in TM mixed with monosaccharides; that is, the decrease in available lysine was 51% in the reaction with glucose for 3 h and 59% in that with ribose for 15 min. On the other hand, the available lysine loss progressed slowly in the reaction with other sugars, and it took 15 days for 48 and 41% of the lysine to be lost in maltose and maltotriose, respectively. In all samples, the production of ketoamine with the loss of available lysine occurred at the early periods of the reaction. The maximum ketoamine content produced in TM was observed in the reaction with glucose for 3 h (346 μmol/g), with ribose for 30 min (529 μmol/g of protein), and with maltose and maltotriose for 4 days (501 and 258 μmol/g, respectively). In addition, the browning of TM occurred in glucose for 48 h, in ribose for 3 h, and in maltose and maltotriose for 15 days (data not shown). These results clearly indicate that TM was modified with the reducing sugars through the Maillard reaction. The reactivity for the TM of the reducing sugars was high, in the following order: ribose > glucose ≫ maltose > maltotriose.

Figure 2 shows the SDS-PAGE patterns of TM-sugars. In all samples, TM decreased markedly, and a broad band having lower mobility simultaneously appeared with the progress of the Maillard reaction. These changes in SDS-PAGE were often observed in the Maillard reaction between protein and reducing sugars (*13*, *23*, *24*). It is apparent that further progress of the Maillard reaction occurred in TM with monosaccharides because a clear band with low mobility was also observed when TM was reacted with glucose for 48 h (71% of the lysine residues were lost) and with ribose for 3 h (68% of lysine was lost).

Effect of Maillard Reaction on Allergenicity of TM. Changes in the allergenicity of TM with the progress of the Maillard reaction were assessed using dot blotting (**Figures 3** and **6**) and competitive ELISA (**Figures 4**, **5**, and **7**) by monitoring the reactivity of TM with the specific IgE antibody in the patients' sera. As shown in **Figure 3**, unreacted (native) TM showed a clear but weak reaction with eight patients' sera. However, the spot intensity of TM became stronger with the progress of the Maillard reaction with glucose. A marked increase in the spot intensity was observed in the reaction for 3 h in patients' sera 1−3 and in that for 12 h in another patients' sera. On the other hand, these enhancements of the spot intensity were not generated when TM-glucose was incubated with control sera ($n = 2$) and fish-allergic patients' sera ($n = 2$) (data not shown). Therefore, it is apparent that the enhanced reactivity shown in **Figure 3** was caused by an increase in the amount of the specific IgE bound to TM-glucose.

Figure 4 shows the result of competitive ELISA in TM-glucose using patients' sera 1−3, which showed strong spot intensity in **Figure 3**. The reactivity of the patients' sera to native TM coated in the microtiter plate gradually diminished with the increase in the concentrations of TM-glucose (reaction for 0−48 h) as inhibitors, and the inhibition rate curves shifted to the left side with the increase in the reaction time. This result indicates that the Maillard reaction with glucose enhanced the

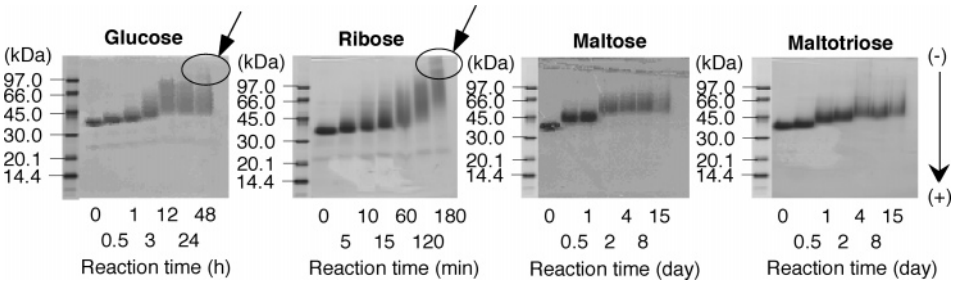

Figure 2. SDS-PAGE patterns of TM reacted with reducing sugars. Arrows show high molecular weight components produced with the progress of the Maillard reaction.

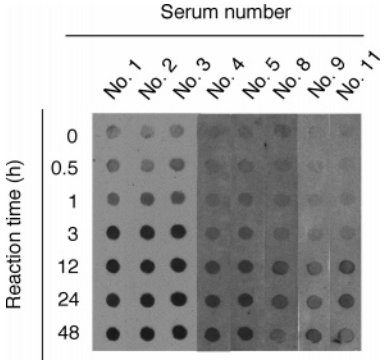

Figure 3. Change in IgE-binding ability of TM during reaction with glucose.

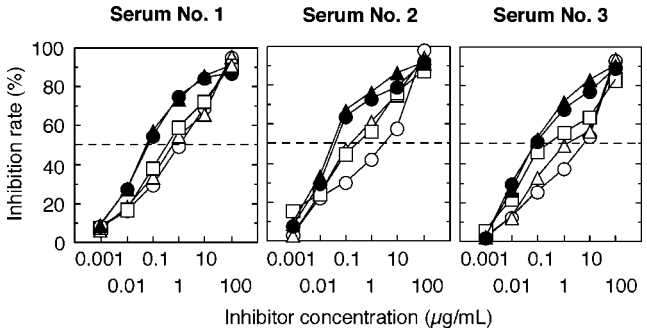

Figure 4. Competitive ELISA of TM-glucose. TMs reacted with glucose for 0 h (native, ○), 1 h (△), 3 h (□), 12 h (●), and 48 h (▲) were mixed with patients' sera 1–3.

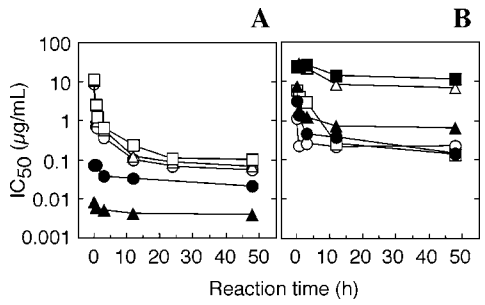

Figure 5. Changes in IgE-binding ability during Maillard reaction between TM and glucose: (**A**) patients' sera 1 (○), 2 (△) 3 (□), 4 (●), and 5 (▲); (**B**) patients' sera 6 (○), 7 (△), 8 (□), 9 (●), 10 (▲), and 11(■).

inhibitory effect of TM in the competitive ELISA. For example, in the case of patient's serum 1, the inhibition rate at 0.1 μg/mL of TM increased from 29 to 57% by the Maillard reaction with glucose for 48 h. Enhancement of the allergenicity in TM-glucose was also observed in all patients' sera (no. 2−11), whereas no change was observed in TM incubated at 60 °C in the absence of glucose (data not shown).

The IC_{50} was then calculated from the inhibition rate curves of competitive ELISA using 11 patients' sera to estimate the enhancement of the allergenicity of TM by the Maillard reaction. A reduction of IC_{50} (i.e., an enhancement of the allergenicity of TM) with the progress of the Maillard reaction was observed in the competitive ELISA using all patients' sera (**Figure 5**). Particularly, >100-fold reductions of IC_{50} were observed in patients' sera 1−3. A marked reduction occurred after 3−12 h of the Maillard reaction, the same period during which the spot intensity in the dot blotting was enhanced (**Figure 3**). Therefore, the structural change of TM that resulted in the enhancement of the allergenicity could have occurred at the early stage of the Maillard reaction.

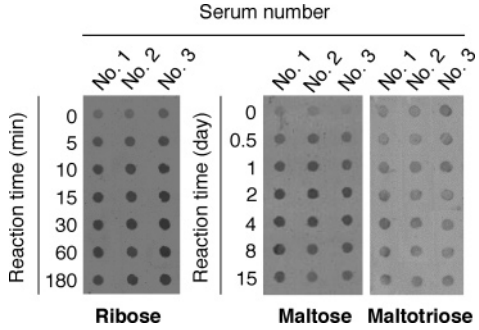

Figure 6. Change in IgE-binding ability of TM during reaction with reducing sugars.

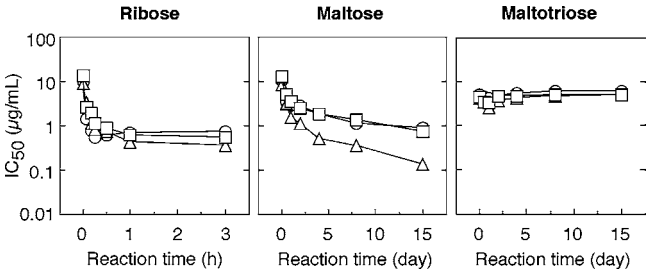

Figure 7. Changes in allergenicity during Maillard reaction between TM and reducing sugars: patients' sera 1 (○), 2 (△), and 3 (□).

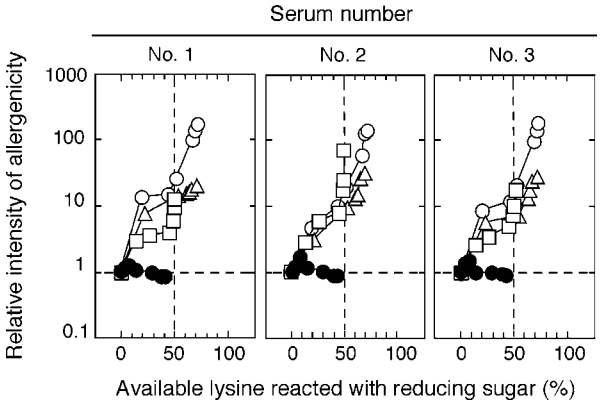

Figure 8. Relationship between allergenicity of TM and available lysine content reacted with various reducing sugars. TM was reacted with glucose (○), ribose (△), maltose (□), and maltotriose (●). The relative intensity of allergenicity was expressed by IC_{50} of native TM as 1.

The allergenicity of TM-ribose, TM-maltose, and TM-maltotriose was assessed by dot blotting using patients' sera 1−3 (**Figure 6**). An enhancement of the spot intensity was observed in TM-ribose and TM-maltose as well as in TM-glucose. In contrast, no change was observed in the spot of TM-maltotriose, even if the lysine loss reached 40%, as shown in **Figure 1**. The change in the allergenicity of TM reacted with the three reducing sugars was also examined in the competitive ELISA, and the changes in the IC_{50} values of TM-sugars are shown in **Figure 7**. The IC_{50} decreased markedly with the progress of the Maillard reaction with ribose and maltose, and 12−25-fold and 15−35-fold increases in allergenicity were, respectively, observed. On the other hand, the reaction with maltotriose had no effect on the allergenicity of TM, as described in **Figure 6**. Therefore, it would appear that the influence of the Maillard reaction differs depending upon the type of reducing sugar used.

Figure 8 shows summarized data concerning the effect of the Maillard reaction on the allergenicity of TM. The relative

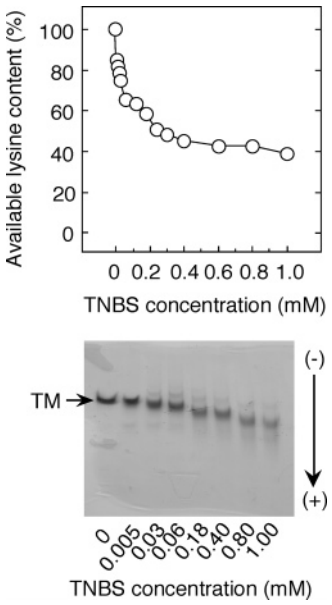

Figure 9. Effect of TNBS concentration on available lysine content of TM and urea-PAGE analysis.

intensity of the allergenicity was plotted against the reaction rate of available lysine in each reducing sugar. The allergenicity was enhanced ~10 times when 50% of the lysine residues were reacted with glucose, ribose, and maltose. In addition, a further enhancement of the allergenicity occurred in the reaction with glucose. In the sera of patients' 1 and 3, the enhancement of the allergenicity of TM tended to weaken as the molecular size of the reducing sugars increased. Thus, it is concluded that glucose is the most effective sugar to enhance the allergenicity of TM through the Maillard reaction.

Effect of TNBS Treatment on Allergenicity of TM. To discuss the relationship between the enhancement of the allergenicity and the lysine loss, TM was modified by TNBS. When TM was reacted with 0.005−1.0 mM TNBS at 30 °C for 3 h, a decrease in the available lysine content and an increase in the mobility of TM in urea-PAGE were observed simultaneously with an increase in the TNBS concentration (**Figure 9**). These results clearly indicate that TNBS was reacted with the lysine residues in TM. The available lysine content decreased to 50% of the initial value when 0.24 mM TNBS was reacted with TM. From the data of **Figure 1**, such a lysine loss level corresponded to the reaction with glucose for 3 h and to that with ribose for 10 min. Because some reduction in the lysine content was observed in the TNBS concentration from 0.24 to 1.0 mM (eventually, the available lysine content decreased to 39%), most of the reactive lysine residues existing at the surface of TM could be modified by the TNBS treatment.

TMs modified with TNBS (TM-TNBS) were subjected to competitive ELISA, and IC_{50} was calculated and plotted against the lysine content reacted with TNBS. In the competitive ELISA, no change was observed in the inhibition curve of TM by the progress of TNBS treatment, and the IC_{50} of TM-TNBS (the modified lysine rate: 61%) almost coincided with that of native TM (**Figure 10**). In other words, the TNBS treatment had no effect on the allergenicity of TM. Because the molecular weight of TNBS was almost equal to that of maltose, the enhancement of the allergenicity that occurred by the Maillard reaction could be independent of the conjugation of lysine residues with reducing sugars and the loss of the positive surface charge of TM.

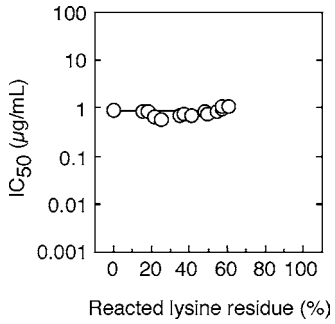

Figure 10. Allergenicity of TM reacted with TNBS.

The results of this study have revealed that the IgE-binding ability of TM was enhanced by the Maillard reaction with reducing sugars. A significant allergenic change was observed at the early stage of the Maillard reaction, regardless of the type of reducing sugar used. Additionally, it was confirmed in the TNBS treatment that the enhancement of the allergenicity of TM-sugars is not related to the protein surface charge. Thus, the allergenic change would be caused by the protein structural change occurring at the early stage of the Maillard reaction. Indeed, the structural change was confirmed by chymotryptic digestion (i.e., the rate of digestibility and the pattern of digestive fragments of TM were altered by the Maillard reaction), whereas the TNBS treatment has little effect on the digestibility of TM (data not shown). However, the increasing rate of the allergenicity was different among the reducing sugars, as described in **Figures 5** and **7**, and no allergenic change was observed in TM-maltotriose. Therefore, details of the structural change that causes the enhancement of the allergenicity of TM need to be examined in more depth. Moreover, the allergenicity changes during the Maillard reaction in the TM of other species as well as scallop TM should be investigated because TM is the major allergen in marine invertebrates (*25*), including shrimp, crab, squid, and octopus.

ABBREVIATIONS USED

BSA, bovine serum albumin; DEAE, diethyl-aminoethyl; ELISA, enzyme-linked immunosorbent assay; IgE, immunoglobulin E; ME, mercaptoethanol; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; PMSF, phenylmethanesulfonyl fluoride; PVDF, poly(vinylidene fluoride); CAP-RAST, capsulated hydrophilic carrier polymer-radioallergosorbent test; SDS, sodium dodecyl sulfate; TBS, Tris-buffered saline; TM, tropomyosin; TNBS, 2,4,6-trinitrobenzenesulfonic acid sodium salt dihydrate; Tween 20, poly(oxyethylene) sorbitan monolaulate.

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