

Allergenicity of acrolein-treated shrimp tropomyosin evaluated using RBL-2H3 cell and mouse model

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Abstract

BACKGROUND: Food processing effects can modify protein functional properties. However, protein was oxidized inevitably by lipid peroxidation during food processing. Acrolein, a primary by-product of lipid peroxidation, can modify the structural and functional properties of protein. The aim of the research was to analyze the effect of acrolein on allergenicity of TM, a major allergen in shrimp.

RESULTS: The overall allergenic effects of acrolein-treated TM were evaluated using female BALB/c mice and a mediator-releasing RBL-2H3 cell line. Acrolein-treated TM significantly decreased TM-specific immunoglobulin E/G1 levels, and histamine and mMCP-1 release in mouse serum. Release of inflammatory mediators such as β -hexosaminidase, histamine, cysteinyl leukotriene and prostaglandin D₂ was clearly suppressed after acrolein treatment.

CONCLUSION: These results indicate that acrolein-induced tropomyosin modification can decrease the allergenicity of TM. This reduction contributes to allergenic potential changes in shrimp during processing and preservation.

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Keywords: shrimp; acrolein; tropomyosin; protein oxidation; allergenicity

INTRODUCTION

Shrimp is a popular seafood with high nutritional value consumed all around the world. However, due to an increase in shrimp-induced allergic incidents, food safety has become a major global concern. Allergic reactions from shrimp pose a serious negative impact on the health of allergic patients.¹ Adverse allergic reactions often result in mucocutaneous (95.7%), respiratory (23.9%), gastrointestinal (16.3%), anaphylactic (11.9%) and cardiovascular (3.3%) symptoms.²

Several attempts to eliminate or reduce the allergenicity and to effectively minimize human health hazards using different processing methods have been made previously.^{3–5} Currently available studies show that various food processing technologies could reduce shrimp allergenicity^{6,7} and provide the required technical support to control allergen risk in the food industry. Nevertheless, due to the large number of chemical reactions occurring during food processing, the factors are too complex to elucidate the variation of allergenicity. In fact, most of the firmly established causes of allergic changes are based on speculation, and the mechanisms leading to allergic changes in food processing technology are rarely explored. In addition, the direct evaluation of allergenicity often overlooks other important steps of food processing that may also contribute to the occurrence of allergic reactions, such as mast cell release with allergen stimulation. Moreover, animal experiments are also an important means of allergy evaluation. Therefore, it is important to discuss the changes of shrimp-induced allergenicity during food

processing steps from a molecular level (protein). This will provide proper guidelines for effective control of allergens in seafood processing.

Shrimps contain unsaturated fatty acids that are extremely prone to oxidation during processing and preservation.⁸ Furthermore, the coexistence of protein and unsaturated fatty acids make protein oxidation inevitable in shrimp products. Uchida⁹ reported that acrolein (CH₂=CH—CHO), one of the most abundant individual aldehyde products formed from lipid peroxidation, is extensively detected in the food system.^{10,11} Acrolein is able to bind to free amines via the formation of Schiff base-type adducts or by addition through the Michael reaction.¹² Such chemical modifications can further induce changes in the conformation and physicochemical properties of proteins.

Tropomyosin (TM), a 36 kDa protein with 284 amino acids, has been described as one of the major allergens in shrimp.¹³ In our previous studies, we explained the effect of the structure and immunoglobulin G/E (IgG/IgE) binding capacity of acrolein-treated TM¹⁴; however, we have not analyzed the

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TM-induced allergenicity in cell or animal models. Previous studies by others were not able to explain with sufficient detail changes in TM-induced allergenicity. Therefore, in this study we compared the allergenicity of TM and acrolein-treated TM by measuring histamine and mMCP-1 release, and determining IgE/IgG1 levels using a BALB/c mouse model. In addition, we also evaluated the allergenicity changes of shrimp TM by measuring β -hexosaminidase and histamine release, and cysteinyl leukotrienes and prostaglandin D₂ levels in an RBL-2H3 cell line using sera from shrimp allergic children. Thus the aim of this study was to investigate the allergic potential of shrimp TM under acrolein effect as an example, and to provide valuable insights for further research into different allergens in various other types of allergenic foods.

EXPERIMENTAL

Materials and reagents

Shrimp (*Metapenaeus ensis*) was purchased alive from a local fish market: Qingdao. Acrolein was procured from Sigma Chemical Co. (St Louis, MO, USA). Alum adjuvant was purchased from Pierce (Rockford, IL, USA). Advanced Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS; certified, heat-inactivated) and penicillin–streptomycin were purchased from Gibco Life Technologies (Grand Island, NY, USA). Enzyme-linked immunosorbent assay (ELISA) kits for histamine, cysteinyl leukotriene, prostaglandin D₂ and mMCP-1 were purchased from R&D Systems (Minneapolis, MN, USA). All other reagents were of analytical grade unless stated otherwise.

Preparation of TM and acrolein-treated TM

Shrimp TM was extracted using the method of Wang *et al.*¹⁵ The oxidation of TM was prepared as follows: TM was incubated with 1 mmol L⁻¹ acrolein in 10 mmol L⁻¹ phosphate buffer (PBS) (pH 7.4) for 24 h at 25 °C. After the incubation, the reaction was stopped by cooling the system to temperatures close to 0 °C in an ice bath. The resulting solution was dialyzed against PBS (pH 7.4) for 24 h at 4 °C to remove the remaining acrolein, and then lyophilized and stored at -80 °C until use. The total protein concentration in each step was monitored using bovine serum albumin (BSA) as a standard.

Serum samples of shrimp-allergic patients

Shrimp-allergic patients' sera were provided by the affiliated hospital of Qingdao University (Qingdao, China). Levels of specific IgE were above 0.5 kUA L⁻¹ (Table 1). The patients had previously described histories of shrimp anaphylaxis, thus indicating obvious symptoms of shrimp exposure. Blood samples were obtained with the written approval from all selected patients and sera were stored at -80 °C until use. The IgE level in the sera was evaluated *in vitro* using ImmunoCAP IgE-specific detection tests (Phadia AB, Uppsala, Sweden). The protocol was approved by the local medical ethics committee (File No. QD-PF-20170913685) to fully protect the rights and interests of human subjects.

Animals and sensitization

Specific pathogen-free (SPF) female BALB/c mice (5 weeks old and weighing 20 ± 2 g) were acquired from Jinan Laboratory at the Animal Center (Jinan, China). The animals were housed under SPF conditions in a room with controlled temperature (23 ± 2 °C) with a 12/12 h light/dark cycle and relative humidity of 55 ± 5%, and allowed open access to food (without shrimp protein in the diet

Table 1. Clinical and serological characterization of shrimp-allergic patients

Number of patients	Age (years)	Sex	TM-specific IgE (kUA L ⁻¹)	Symptom
2365	3	M	7.66	Bronchial asthma
2878	2	F	15	Cough variant asthma
3546	6	M	18.3	Asthma
6386	3	M	25.6	Leukocytosis

A serum with the specific IgE > 0.5 (kUA L⁻¹) is defined as possible. M, male; F, female.

and tap water. All animal experiments were performed following established guidelines and experimental protocols in accordance with the Ethical Committee of Experimental Animal Care at Ocean University of China.

Sensitization to TM by intraperitoneal (i.p.) injection

Each mouse was sensitized with 200 µg TM or acrolein-treated TM ($n = 10$) with 2 mg mL⁻¹ alum adjuvant by i.p. injection on days 7, 14, 21 and 28 as described in Fig. 1(a). In parallel, the normal group was administered, by i.p. injection, a 0.9% saline solution on days 7, 14, 21 and 28 of the experiment. To measure the level of specific antibodies, blood was collected on day 31. TM-specific IgE and IgG1 serum level was evaluated using ELISA, as described previously.¹⁶ Concentrations of histamine and mMCP-1 in sera obtained 1 h after i.p. injection on day 28 were detected using commercially available ELISA assay kits from R&D Systems (Minneapolis, MN, USA).

Allergenicity in RBL-2H3 cells

The rat mast cell line RBL-2H3 was acquired from the Chinese Academy of Sciences and grown in DMEM, supplemented with 10% fetal bovine serum with 100 µg mL⁻¹ streptomycin and 100 U mL⁻¹ penicillin, and incubated at 37 °C with a 5% CO₂/95% air atmosphere following the method of Hoffmann *et al.*¹⁷

RBL-2H3 cells were harvested at the stationary phase, seeded in 96-well plates with 5 × 10⁵ cells, passively sensitized with human serum IgE from shrimp-allergic children for 15 h and further washed three times with Tyrode's buffer. Moreover, RBL-2H3 cells were stimulated with 500 ng mL⁻¹ of either TM or acrolein-treated TM in Tyrode's buffer at 37 °C.¹⁸ Cells treated with only Tyrode's buffer were employed as negative control. β -Hexosaminidase release was measured as described by Kuehn *et al.*¹⁹ and samples were then centrifuged for 10 min and 4 °C to pellet debris and granules. The release of histamine, cysteinyl leukotriene and prostaglandin D₂ was measured in the resulting supernatant by ELISA, following the manufacturer's instructions.

Statistical analysis

Data are depicted as mean ± standard deviation (SD). Differences among experimental data were analyzed by analysis of variance (ANOVA) using SPSS Statistics 18 software (SPSS Inc., Chicago, IL, USA). The statistical significance of each test was defined at $P < 0.05$.

RESULTS AND DISCUSSION

We used SPF female BALB/c mice as an allergy animal model to test the allergenicity of acrolein-treated TM (Fig. 1a), in which

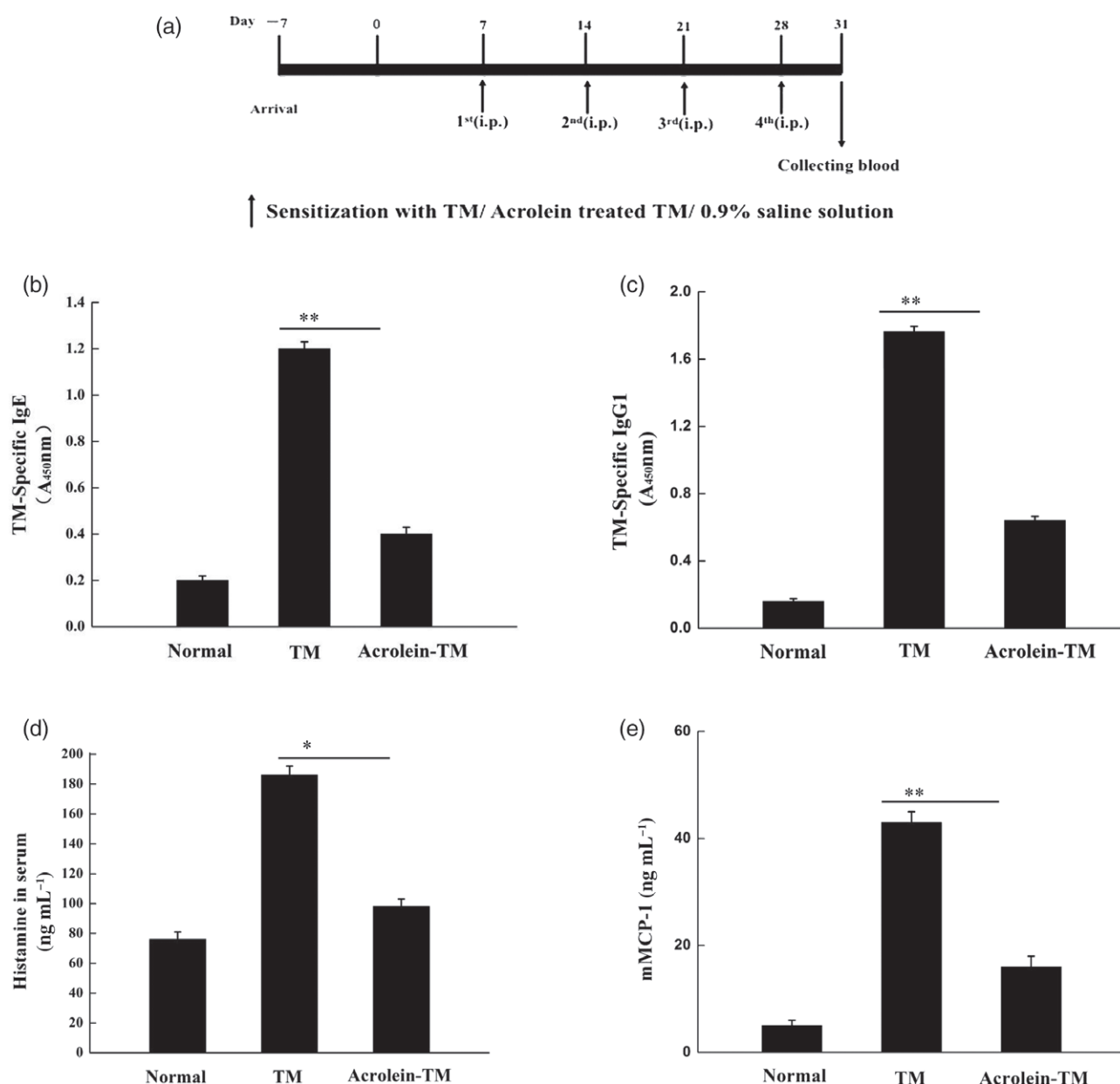


Figure 1. Sensitization experiment. (a) Timeline of the mouse experimental procedure; level of (b) TM-specific IgE and (c) TM-specific IgG1 levels in BALB/c mouse serum measured on day 31; release of (d) histamine and (e) mMCP-1 in BALB/c mouse serum. * $P < 0.05$; ** $P < 0.01$. The data are presented as mean $\pm$ SD from triplicate determinations.

TM-specific IgE and IgG1 serum levels were measured. The immune system produces an allergic response by the release of IgE and IgG1 upon exposure to an allergen. Thus, as shown in Fig. 1(b), TM-sensitized mice had significantly higher ($P < 0.01$) TM-specific IgE levels than the saline-treated normal control group. Notwithstanding, the acrolein-treated TM sensitized group had significantly lower ($P < 0.01$) TM-specific IgE levels than the TM-sensitized groups, which is reflected in a significant decrease (-66.1%) in the release of TM-specific IgE when compared to TM-sensitized mice. In addition, this situation is also observed in the detection of serum levels of TM-specific IgG1 (Fig. 1c). The quantity of histamine and mMCP-1 release in mouse serum increased after sensitization with TM compared to the normal control group. In contrast, histamine and mMCP-1 release decreased significantly ($P < 0.05$) after sensitization with acrolein-treated TM

than the TM-sensitized groups, but still with higher values than the normal control group (Fig. 1d, e). According to our results, the oxidation of acrolein-treated TM changed the sensitization potential of TM in the animals. Allergic sensitization to TM was more prominent when compared to acrolein-treated TM, as shown by a significantly higher production of TM-specific IgG1 and IgE. In general, acrolein-treated TM had a much lower allergenicity response compared to the allergenicity of the native TM. Interestingly, some allergens achieve the same effect of reducing allergenicity due to different chemical modifications. Park *et al.*²⁰ reported that the allergenicity of ovalbumin in chicken egg white decreased after treatment with *N*-acetylglucosaminidase. In addition, Liu *et al.*²¹ reported that enzymatic cross-linking of TM decreased its allergenicity in mice by decreasing the IgE/IgG1 levels in serum. One possible explanation raised for this observation is that the

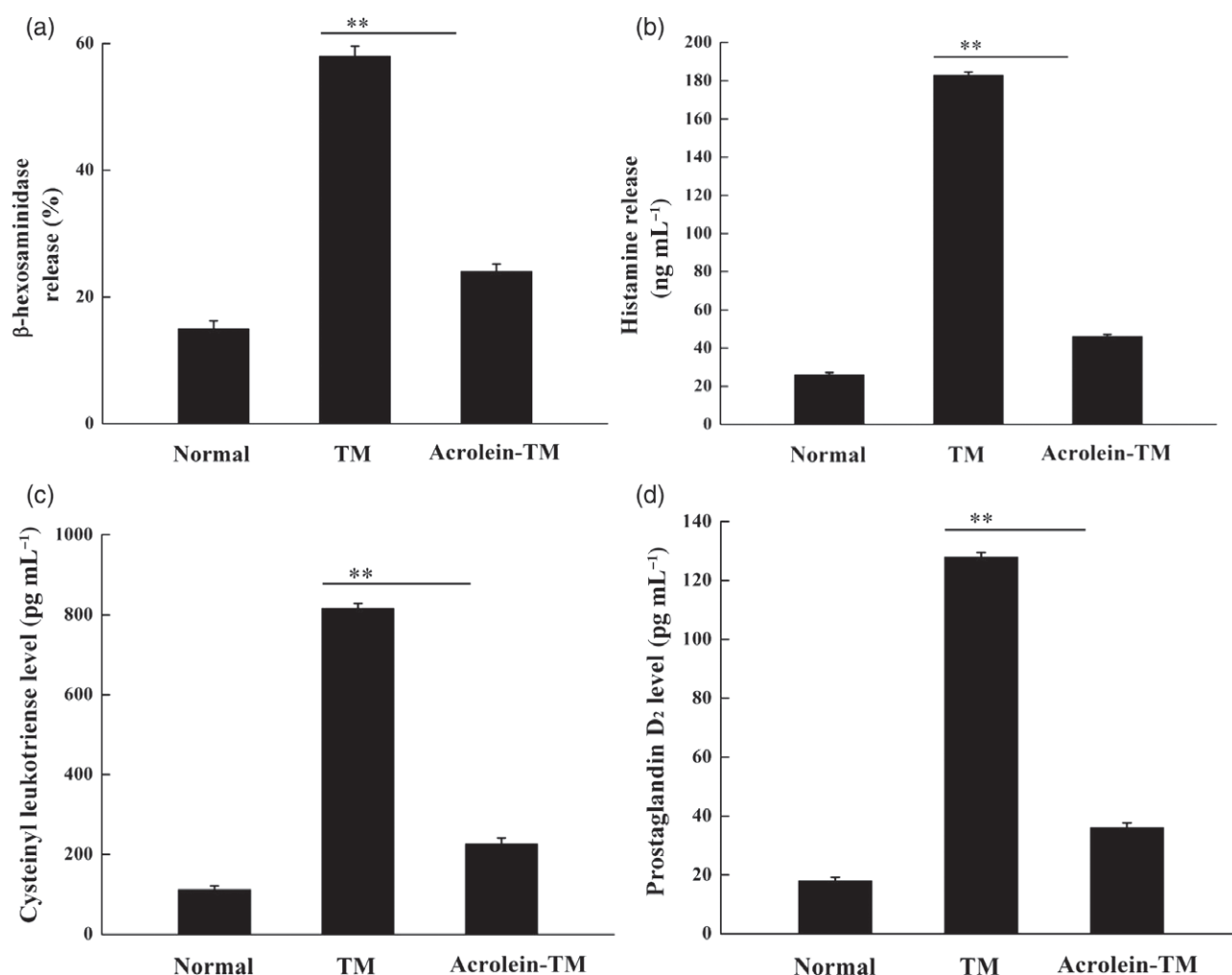


Figure 2. Effects of acrolein-treated TM in IgE-mediated allergic responses in RBL-2H3 cell line. The release of (a) β -hexosaminidase, (b) histamine, (c) cysteinyl leukotrienes and (d) prostaglandin D₂ levels from RBL-2H3 cells sensitized with pool sera from children allergic to shrimp. * $P < 0.05$; ** $P < 0.01$. The data represent the mean $\pm$ SD of three independent experiments.

chemical modification covered or destroyed the IgE epitope and changed the immunological properties *in vivo*, thus successfully decreasing its allergen-inducing capacity.

The allergenicity induced by acrolein-treated TM is similar in the IgE-mediated RBL-2H3 cell assay. Passive rat basophil leukemia (RBL) cells are generally used as models for type I allergic reactions and to establish allergen-specific standards of allergenic potential. Therefore, RBL-2H3 cells (sensitized with shrimp allergic patient's sera) were employed to investigate the influence of acrolein oxidation on shrimp TM allergenicity by monitoring the release of β -hexosaminidase, histamine, cysteinyl leukotriene and prostaglandin D₂ (depicted in Fig. 2). After sensitizing with each TM variant, RBL-2H3 cells released a marker of mast cell degranulation: β -hexosaminidase. TM induced substantial degranulation of RBL-2H3 cells, and acrolein-treated TM decreased the level of degranulation ($P < 0.01$) compared to TM (Fig. 2a). Acrolein-treated TM also aids in the inhibition of antigen-induced histamine release in RBL-2H3 cells (Fig. 2b), an effect that is also observed for the other allergenic mediators (cysteinyl leukotriene and prostaglandin D₂). In general, the data indicate that TM oxidation may have significant effects on the suppression of the release of mediators related to allergic symptoms. This was in agreement with a previous study in which

the treatment of TM with MDA decreased degranulation.²² The proposed explanation was that the response of RBL-2H3 cells to the allergen by oxidation may be due to altered IgE epitopes on the surface of acrolein-treated TM, hindering successful basophil degranulation. The protein modification may alter its allergenic potential due to changes in the epitope region.

Both mouse model and RBL-2H3 cell assay revealed that acrolein-treated TM decreased allergenicity. This may be explained by the covering of epitopes in TM. Epitopes of TM with IgE affinity were described previously.²³ In previous research performed by our team, we also showed that amino acid residues of TM with acrolein were primarily present in the side chains of lysine, tyrosine and histidine by liquid chromatography–tandem mass spectrometry (LC–MS/MS), affecting the conformational (secondary and tertiary) structure. In addition, we also found that TM oxidation decreased IgG/IgE binding, supported by data from both animal and cell studies. The research discovered that the amino acid residues of lysine, tyrosine and histidine in TM linear epitopes which react with acrolein led to a reduction in IgE-binding activity. TM is a protein in which linear epitopes are dominant.²³ Acrolein may react with the linear epitopes in TM. The destruction of a linear epitope made decrease allergenicity. The study further

illustrated that acrolein oxidation can change the structure of TM, hide the epitopes of TM and decrease the allergenicity of TM in shrimp.

CONCLUSION

The results presented in this study indicate that acrolein-treated TM has the potential to mitigate allergenicity. Oxidation elicited slight allergic sensitization in mice, resulting in a decrease in specific IgE response. The reduction in allergenicity of acrolein-treated TM was also determined using an RBL-2H3 cell assay. These changes indicated that the allergenic potential of shrimp TM might be affected during food processing and preservation. Thus acrolein-treated TM might be used as an immunotherapeutic agent against the TM allergen, and the agent could also be used to check the allergenicity of shrimp products. Further studies should be conducted in order to elucidate which molecular factors are modulated by acrolein-treated TM at the cell membrane receptor level and in the control of gene expression of allergenic mediators.

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