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Changes of structure and IgE binding capacity of shrimp (*Metapenaeus ensis*) tropomyosin followed by acrolein treatment†

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Tropomyosin, a major allergen of shrimp, presents high stability to various conditions owing to the high content of α -helix. Chemical modification of tropomyosin can induce structural changes and affects IgE binding capacity. Since lipid peroxidant such as acrolein commonly exists during shrimp preservation, it is necessary to evaluate its influence on tropomyosin structure and IgE binding capacity. Western blot and inhibition enzyme-linked immunosorbent assay were used to illustrate IgE binding capacity, while structural changes were investigated by liquid chromatography-tandem mass spectrometry (LC-MS/MS) and fluorescence spectroscopy. Results revealed that IgE binding capacity reduced and the secondary structure denatured following treatment with increasing concentration of acrolein. Furthermore, the identification of LC-MS/MS indicated that the free amines of lysine, tyrosine, and histidine residues were altered by acrolein. These amino acid modifications resulted in the collapse of epitopes, as well as structural changes responsible for the decreased IgE binding capacity of shrimp tropomyosin.

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1. Introduction

Shrimp is a popular aquatic product that is used in many kinds of food because of its tasty flavour and rich nutrition. However, it is also an important food allergen, which is responsible for many allergic diseases.^{1,2} Individuals sensitized to shrimp are advised to avoid dietary shrimp based on the presence of shrimp-specific IgE antibody in their sera. Specific IgE has been reported to play a key role in the context of IgE-mediated shrimp allergy.³ Therefore, it is necessary to evaluate the changes in IgE binding capacity of shrimp allergen during food processing, as shrimps are mostly served after processing.

Although several researchers have reported the influence of food processing on shrimp allergens,^{4,5} their structure and immunologic characterizations have not been studied as extensively as other food allergens such as peanuts. Furthermore, as a kind of aquatic product, shrimp contains high contents of unsaturated fatty acids and is extremely prone to oxidation during processing and preservation.⁶ Many works have reported that proteins are mostly affected by reactive oxygen

species (ROS) which are created due to oxidation of lipids.^{7–9} Coexistence of protein and unsaturated fatty acids makes protein oxidation inevitable in shrimp products. Recently, Uchida¹⁰ reported that acrolein ($\text{CH}_2=\text{CH}-\text{CHO}$), one of the most abundant aldehydes formed from lipid peroxidation and existing extensively in the food system,^{11,12} could bind with free amines *via* Michael addition reaction or formation of Schiff base-type adducts. These modifications can further induce changes in conformation and physicochemical properties. Based on this, it was hypothesized that such oxidative modifications could definitely influence the IgE binding capacity of shrimp allergens.

Tropomyosin, known to be the major allergen, contributes 5–7% of shrimp muscle,¹³ and can cause a reaction in at least 80% of shrimp-allergic patients.¹⁴ The epitopes of tropomyosin are responsible for the allergic activity. Ayuso *et al.*¹⁵ and Shanti *et al.*¹⁶ reported that there are eight major IgE binding regions, which are the basis of IgE binding capacity of tropomyosin. Further analysis showed that these epitopes contain plenty of amino acid residues with free amines, such as lysine, histidine, and tyrosine.¹⁷ These free amines react easily with the aldehyde group in acrolein and cause alterations in protein structures. In our previous work, we have demonstrated that malondialdehyde, another carbonyl compound, with double aldehydes formed from lipid peroxidation, could modify the structure and IgE/IgG binding capacity of shrimp tropomyosin through binding with its amino acid residues.^{18,19} However,

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knowledge of the effect of lipid peroxidants on the IgE binding potential of shrimp tropomyosin is still very limited.

This research work was performed in order to ascertain whether the reaction of acrolein with shrimp tropomyosin could induce structural transformation, thereby altering the IgE binding capacity. In addition, the sites of tropomyosin modification were identified to illustrate the possible mechanism of the IgE binding capacity changes. This work helps to explain the changes of shrimp allergic properties caused by lipid oxidation during shrimp processing and preservation.

2. Materials and methods

2.1 Materials and chemicals

Acrolein, 3,3',5,5'-tetramethylbenzidine (TMB), bovine serum albumin (BSA) and 1-anilino-8-naphthalene-sulfonate (ANS) were procured from Sigma Chemical Co. (St Louis, MO, USA), while anti-rabbit IgG and horseradish peroxidase (HRP)-conjugated goat anti-human IgE antibodies were obtained from Zhongshan Jinqiao Co. (Beijing, China). The enhanced chemiluminescence (ECL) substrate used in western blot analysis was procured from Thermo Fisher Scientific Inc. (Waltham, MA, USA). The protein standard for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was procured from Solarbio Co. (Beijing, China). Purified shrimp tropomyosin and rabbit serum against tropomyosin were obtained from our laboratory. All other chemical reagents used were of analytical grade.

2.2 Human sera

Sera from shrimp-allergic patients were provided by Qingdao Municipal Hospital (Qingdao, P. R. China). The patients signed written consent forms before collecting sera in order to ensure their knowledge about the usage of their sera. The patients were diagnosed using skin prick test. Sera having a specific IgE level above 0.5 kUA L⁻¹ were selected by Allergy Screen (MEDIWISS Analytic GmbH, Moers, Germany) and then kept at -40 °C until use. The Food Safety Committee, College of Food Science and Engineering, Ocean University of China approved the sera collection protocol (File no. QD-PF-20160516316).

2.3 Reaction of tropomyosin with acrolein

Acrolein solution was freshly prepared by dilution in phosphate buffer (PB) (10 mM, pH 7.4). Shrimp tropomyosin samples (10 mg mL⁻¹) in the same PB solution were mixed with acrolein solution at an equal ratio and incubated at 25 °C for 24 h. According to previous research on the content of acrolein in food, the concentration of acrolein ranges from micromolar to millimolar. In this work, the concentrations 0 (control), 0.01, 0.1, 0.5, and 1 mmol L⁻¹, respectively were used.^{20,21} After incubation, the reaction system was cooled to 0–4 °C by immersing in an ice bath.

2.4 SDS-PAGE analysis

Acrolein-treated tropomyosin samples were subjected to SDS-PAGE according to the method described by Laemmli.²²

The samples (8 µg per well) were mixed well with bromophenol blue-buffer (4:1) and were heated for 7 min at 100 °C. SDS-PAGE gel was prepared with 5% stacking gel and 12% running gel. Electrophoresis of the proteins was performed in the stacking gel at 80 V for 0.5 h and in the resolving gel at 120 V for 1.5 h. Coomassie Brilliant Blue R-250 (Solarbio, China) was used to stain the gels, followed by destaining until the solution became clear. Protein bands were determined by using Tanon-4200SF gel imaging and analysis software (Tanon Science and Technology Co., Ltd, Shanghai, P. R. China).

2.5 Immunological characteristics of acrolein-induced tropomyosin

2.5.1 Western blot. Samples were separated with SDS-PAGE and electrophoretically transferred onto 0.22 mm pore polyvinylidene fluoride (PVDF) membrane.²³ The membrane was incubated at 4 °C overnight in blocking buffer and then washed with PBST (phosphate-buffered saline containing Tween 20) three times. It was then incubated with IgE (human serum, diluted 1:8) or IgG (rabbit serum, diluted 1:1000) and washed three times using PBST. The membrane was then incubated with anti-IgE (diluted 1:200) or anti-IgG (diluted 1:5000) as the secondary antibody. The results were detected by ECL after the membrane had been extensively washed with PBST.

2.5.2 Competitive inhibition ELISA (ciELISA). The specific IgE binding activity of protein was analysed through competitive inhibition enzyme-linked immunosorbent assay (ciELISA) according to the method of Lasekan & Nayak.²⁴ The purified tropomyosin was dissolved in carbonate coating buffer (100 mL, 0.1 M, pH 9.1) at a concentration of 5 µg mL⁻¹, and was introduced into Micrilon ELISA plates and then incubated at 4 °C for 12 h. In another plate the concentration of tropomyosin varied from 0.025 µg mL⁻¹ to 100 µg mL⁻¹. PBST was used to rinse the coated plates, which were then incubated in the blocking buffer (containing 5% skimmed milk) for 2 h at 37 °C. Fifty microlitres of antisera (shrimp-allergic patients' sera diluted 1:2 with 2.5% skimmed milk in PBST) was subsequently incubated with the acrolein-treated protein (1:1) at 37 °C for 1.5 h. Fifty microlitres of the mixture was blended with the purified tropomyosin and incubated in the ELISA plates for 1.5 h at 37 °C. After being incubated with the secondary antibody, the mixture was reacted with TMB. An automated ELISA plate reader (Multiscan MK3; Thermo Fisher Scientific Inc.) was used to determine the absorbance of the mixture at 450 nm. The percentage inhibition was determined in order to evaluate the inhibition rate according to the following formula:

$$\text{Inhibition rate (\%)} = \frac{[\text{OD no inhibitor} - \text{OD inhibitor}]}{[\text{OD no inhibitor}]} \times 100\%$$

2.6 Structure analysis

2.6.1 Surface hydrophobicity. Analysis of the surface hydrophobicity of protein was performed using ANS as fluorescent probe, as reported by Huang *et al.*²⁵ The samples were dissolved in PB (0.01 M, pH 7.4) at a concentration of 1 mg mL⁻¹.

Serial dilution of the solution was performed to the final concentrations of 0.005–0.5 mg mL⁻¹. Forty microlitres of ANS solution (5 mM, in 0.01 M PB, pH 7.4) was mixed with 2 mL protein solution to react for 3 min. The intensity of the fluorescence was measured at 365 nm (excitation wavelength) and 484 nm (emission wavelength) using a fluorescence spectrophotometer (Hitachi Ltd, Tokyo, Japan).

2.6.2 Intrinsic fluorescence. The intrinsic fluorescence of all the samples (20 mg mL⁻¹) in PB (0.01 M, pH 7.4) was measured at 25 °C by using an RF-5301PC fluorescence spectrometer (Shimadzu, Kyoto, Japan), as reported by Wu and co-workers.²⁰

2.6.3 UV absorption spectra. The UV absorption spectra of all the samples (0.5 mg mL⁻¹) were scanned in the 230–310 nm range and then analysed using the UV-1101 system from Techcomp (Shanghai, P. R. China).

2.6.4 Circular dichroism spectroscopy analysis. Circular dichroism (CD) spectroscopy was performed in order to define the secondary structure of the original and oxidatively modified tropomyosin. The experiment was conducted with a JASCO J-815CD spectropolarimeter (Japan Spectroscopic Co. Inc., Japan).²⁶ The spectra were scanned at 190 to 260 nm. A protein concentration of 1 mg mL⁻¹ was used for analysis. The operation indexes are as follows: scan speed 100 nm min⁻¹; bandwidth 1 nm; response time 2 s. The average results of the molar ellipticity of residues were evaluated and expressed as deg cm² dmol⁻¹. Then, we used JASCO software to calculate the content of various secondary structures.

2.7 Oxidation site analysis

According to the reports of Lv *et al.*¹⁹ the method of LC-MS-MS was conducted as follows. The protein samples were subjected to SDS-PAGE electrophoresis. Target proteins were fragmented into different peptides and put into eppendorf (EP) tubes with 50 µL double distilled water (DD). Target proteins were washed twice for 10 min using deionized H₂O. Fifty microlitres of a solution containing 50% (v/v) NH₄HCO₃ and 50% (v/v) acetonitrile was added to 50 µL of deionized H₂O solution and bleached at 37 °C for 20 min. The samples were blotted up until the blue colour faded. Particles were completely dehydrated with acetonitrile (50 µL) and blotted up in a vacuum for 10 min. Then, 10 mmol DTT [1% (v/v) DTT and 99% (v/v) NH₄HCO₃] was added in 20 µL solution and incubated for 1 h at 56 °C. The samples were blotted up after cooling to room temperature. Then 55 mmol iodoacetamide (IAM) [5.5% (v/v) IAM and 94.5% (v/v) NH₄HCO₃] in 20 µL solution was quickly added, and the mixture placed in a dark room for 45 min. The samples were successively washed twice with 25 mM NH₄HCO₃, 25 mM NH₄HCO₃ plus 50% acetonitrile, and acetonitrile for 10 min. Particles were completely dehydrated and blotted up in the same way. The trypsin solution (0.1 µg µL⁻¹) was diluted 15 times using 25 mM NH₄HCO₃ and then mixed fully with the particles by means of slight centrifugation. The solution was placed at 4 °C for 30 min until completely absorbed by the particles. Then 25 mM NH₄HCO₃ was added to a total volume of 10–15 µL at 37 °C and digestion took place

overnight. TFA (1%) was used to stop the reaction, and finally the TFA was diluted to 0.1%. A Q-Exactive mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) with a nanoelectrospray ionization (ESI) source was used for peptide analysis. The peptide samples were separated by high performance liquid chromatography (HPLC) (Eksigent, Dublin, CA, USA). Mobile phase A was deionized water containing 0.1% (v/v) formic acid, while mobile phase B was composed of acetonitrile with 0.1% (v/v) formic acid. The concentration of mobile phase B was raised linearly from 5 to 30% B in 30 min and then to 60% B for 5 min, and finally to 5% B for re-equilibration. Then the eluted peptides were detected by mass spectrometry.

2.8 Statistical analysis

We used analysis of variance (ANOVA) to test the statistical significance of the data by means of SPSS 15.0 software (SPSS Inc., Chicago, IL, USA). Each experiment was performed in triplicate to ensure reproducible results. Values of (*P* < 0.05) were considered significant.

3. Results and discussion

3.1 SDS-PAGE analysis of tropomyosin

The analysis of tropomyosin was performed by SDS-PAGE following treatment with different concentrations of acrolein (Fig. 1). The band of tropomyosin appeared corresponding to a molecular weight of 36 kDa (Fig. 1, lane 0).²⁷ Overall, the protein bands remained unchanged with acrolein concentrations below 1 mM (Fig. 1, lanes 1 and 2). However, when the concentration of acrolein was above 1 mM, a new band at approximately 72 kDa was seen (Fig. 1, lane 3). In addition, the intensity of the tropomyosin band at 36 kDa decreased as compared with control and other samples having lower concen-

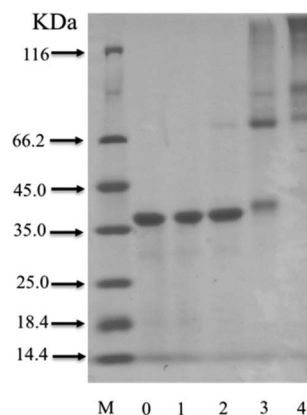


Fig. 1 Sodium dodecyl sulfate–polyacrylamide gel electrophoresis analysis of acrolein-treated shrimp tropomyosin. The molecular mass range of the standard protein marker was from 14.4 to 116 kDa. Lane M: protein marker; Lane 0: shrimp tropomyosin; Lane 1: 0.01 mM acrolein; Lane 2: 0.1 mM acrolein; Lane 3: 1 mM acrolein; Lane 4: 10 mM acrolein. An equal amount of tropomyosin (5 µg) was loaded into each lane.

trations of acrolein. As the acrolein concentration increased, new bands appeared. For instance, new bands at approximately 100 kDa and above were observed when tropomyosin was treated with an acrolein concentration of 10 mM (Fig. 1, lane 4). These data demonstrated that the cross-linking of tropomyosin occurred on treatment with 1 mM acrolein and the cross-linking increased due to higher concentrations of acrolein. The possible reason is that acrolein possesses an aldehyde group and a double bond. These groups can combine with two or more proteins and cause cross-linking. Similarly, the consequence of cross-linking induced by acrolein is supported by a report about soy protein.²⁰

3.2 Determination of IgG/IgE binding capacity

IgG/IgE binding capacity of tropomyosin with acrolein treatment was detected through western blot. As shown in Fig. 1, after acrolein treatment tropomyosin aggregates were visualized by SDS-PAGE. These high molecular weight aggregates contain tropomyosin components, as indicated by their binding to IgG and IgE (Fig. 2A). The binding intensity decreased as an effect of higher concentrations of acrolein. In addition, the IgE binding capacity was remarkably reduced after treatment with increasing concentrations of acrolein. Furthermore, the cross-linking was detected by western blot and the results illustrated that the epitopes of tropomyosin were still active when the concentration of acrolein was more than 1 mM. Song *et al.*¹⁸ claimed that the IgE binding potential of tropomyosin decreased with treatment by increasing

concentrations of malonaldehyde (MDA). A comparable trend was also observed in this study with regard to the potential IgE binding capacity of tropomyosin, using pooled sera from different patients. One possible reason for this phenomenon is that the modification of IgG/IgE binding epitopes that had occurred offered fewer binding sites for these antibodies.

Competitive inhibition ELISA was used to analyse the IgE binding capacity of tropomyosin. The IgE binding capacity was remarkably decreased when tropomyosin was treated with 0.01 and 0.1 mM acrolein, and the decrease was more drastic at 1 and 10 mM, as shown in Fig. 2(B). The IC₅₀ value increased from 0.075 to 0.09, 0.16, 5.8, and 16 $\mu\text{g mL}^{-1}$ with the increase of acrolein concentrations from 0.01 to 0.1, 1, and 10 mM, respectively, compared with the control. These results indicated that acrolein treatment had a great influence on the IgE binding capacity. This might be due to alteration in the side-chains of amino acids leading to the destruction of certain epitopes and reduction of IgE binding capacity.

3.3 Structural change of acrolein-treated shrimp tropomyosin

Analysis of surface hydrophobicity was used to assess the conformational changes. The tropomyosin surface hydrophobicity was altered by increasing the concentration of acrolein. As shown in Fig. 3(A), when tropomyosin was treated with 0.01 mM acrolein, the surface hydrophobicity rapidly increased. The changes were more and more obvious with the increase of the acrolein concentration. Wu *et al.*²⁸ found that the increase of protein oxidation and surface hydrophobicity occurred simultaneously with the addition of malondialdehyde (MDA). The increase in the surface hydrophobicity indicates that hydrophobic residues, which originally should be folded and packaged to form the tertiary structure of the protein, now are exposed to the outside.²⁹ We confirmed that this could be the reason why acrolein treatment caused changes in IgE binding capacity.

The intrinsic fluorescence intensity, as the main structural characteristic of protein, was used to assess the conformational changes. The intrinsic fluorescence spectra of proteins is mainly due to fluorescence emission by tryptophan (Trp) and tyrosine (Tyr) residues. However, shrimp tropomyosin contains no Trp;³⁰ therefore, only Tyr influences the intensity of fluorescence. Emission was seen at 312 nm for excitation at 280 nm. The intrinsic fluorescence intensity of tropomyosin with increasing acrolein concentration is shown in Fig. 3(B). After being incubated with increasing concentrations of acrolein, the maximum Tyr fluorescence intensity decreased and the maximum emission wavelength (from 311 to 308 nm) underwent a blue shift. The intrinsic fluorescence intensity of tropomyosin treated with 10 mM acrolein for 24 h was approximately 17% of the control. Tyr fluorescence intensity decreased as a result of the change of the microenvironment where Tyr residues were combined with the acrolein. Moreover, the blue shift indicates that Tyr residues were buried in the interior of the tropomyosin molecule upon acrolein treatment. The changes illustrate that the modification of

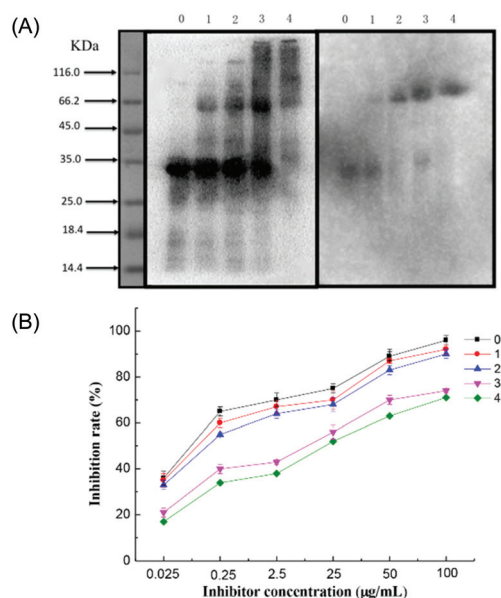


Fig. 2 Analysis of the antigenicity of acrolein-treated tropomyosin. (A) IgG/IgE binding for detecting changes in the antigenic activity of acrolein-treated tropomyosin, using pooled sera from patients allergic to shrimp. (B) Inhibition enzyme-linked immunosorbent assay (ELISA) for detecting changes in the antigenic activity of acrolein-treated tropomyosin, using the pooled sera of the patients in (A). Lanes 0–4 represent the acrolein concentrations 0, 0.01, 0.1, 1, and 10 mM, respectively.

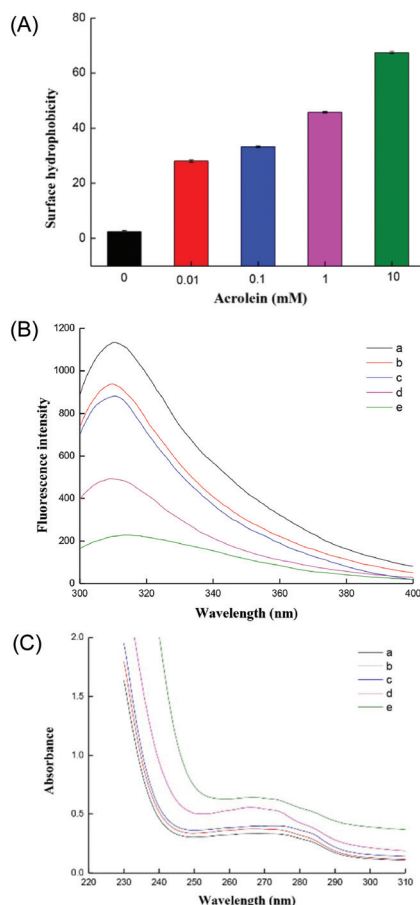


Fig. 3 Effect of increasing concentrations of acrolein treatment on the (A) surface hydrophobicity, (B) intrinsic fluorescence, and (C) UV absorption of native (untreated) and treated tropomyosin in phosphate buffer. a: control (untreated); b: 0.01 mM acrolein; c: 0.1 mM acrolein; d: 1 mM acrolein; e: 10 mM acrolein. In (A), bars with different concentrations are significantly different ($P < 0.05$). In (C), the main absorption peaks were observed between 230 and 310 nm.

the conformational structure of the protein led to aggregation, and thus surface hydrophobicity.

We can infer conformational changes *via* the changes in UV absorption of proteins. The UV absorption spectrum of the native protein is based mostly on Trp and Tyr residues in the side-chain groups.³¹ As with fluorescence, the absence of Trp in shrimp tropomyosin also means that Tyr plays a very important role in UV absorption. The highest absorption peak of tropomyosin appeared at about 275 nm and decreased because of the modification of Tyr residues on the protein molecule surface by the acrolein treatment (Fig. 3C). Furthermore, a minor blue shift was apparent in the 0.1, 1 and 10 mM acrolein-treated samples, which shows that changes of the polarity of the Tyr microenvironment occurred after oxidation, as the peptide bonds were broken. The conformational change of tropomyosin with acrolein treatment may be the main reason for the decrease of its potential IgE binding capacity.

Far-ultraviolet circular dichroism (CD) spectroscopy of a protein could reflect its secondary structure directly. Therefore,

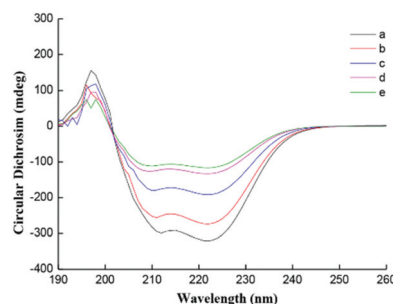


Fig. 4 Far-ultraviolet circular dichroism spectroscopy of acrolein-treated tropomyosin. a: Control; b: 0.01 mM acrolein; c: 0.1 mM acrolein; d: 1 mM acrolein; e: 10 mM acrolein.

we analysed native and acrolein-treated tropomyosin by CD spectroscopy. According to Li *et al.*³² the secondary structure of protein comprises α -helix, β -sheet, β -turn, and random structure. The spectrum of the native tropomyosin produced a positive peak (~ 196 nm) and two negative peaks (~ 212 nm and 223 nm) (Fig. 4). The positive peak represents the β -sheet of tropomyosin, and the negative peaks represent the presence of the α -helix. By increasing the concentration (0.01 – 10 mmol L^{-1}) of acrolein treatment, the intensity of the negative peaks at 212 and 223 nm, and the positive peak at 196 nm steadily decreased. The results meant the presence of α -helical structure was diminished and β -sheet was gradually lost, which indicated the structure became considerably disordered with increasing acrolein treatment. According to the study of McClain *et al.*,³³ this phenomenon might be attributed to acrolein breaking the original van der Waals interactions between the amino acid residues. Such changes disrupted the secondary structure and then rearranged it to a certain extent. In general, the unfolding of protein could lead to the exposure of antigen epitopes.³⁴ In summary, acrolein treatment decreased tropomyosin IgE binding capacity by damaging its conformational epitopes, which also changed the tertiary structure of the protein, inducing aggregation.³⁵

3.4 Identification of acrolein adduction sites

A proteomics approach was used for the analysis of the sites of the tropomyosin modified by acrolein treatment. Based on the results of SDS-PAGE analysis of acrolein-treated tropomyosin (Fig. 1), the protein bands were excised and subjected to further analysis by LC-MS/MS.

According to the principle of Schiff base formation, reactions of amino acid residues of tropomyosin with acrolein occurred primarily in the side-chains of lysine (Lys), tyrosine (Tyr), and histidine (His). An increment of 38 Da was detected with Schiff base adducts by a Mascot search. In addition, methionine (Met) residues were found with an increment of 16 Da. These results indicated reactive oxygen [O] addition during the oxidation of Met. Similarly, other researchers have also reported that Met was oxidized through reactive oxygen species.³⁶ As shown in Table 1, a total of 15 peptides reacted with acrolein and reaction took place in the form of Schiff

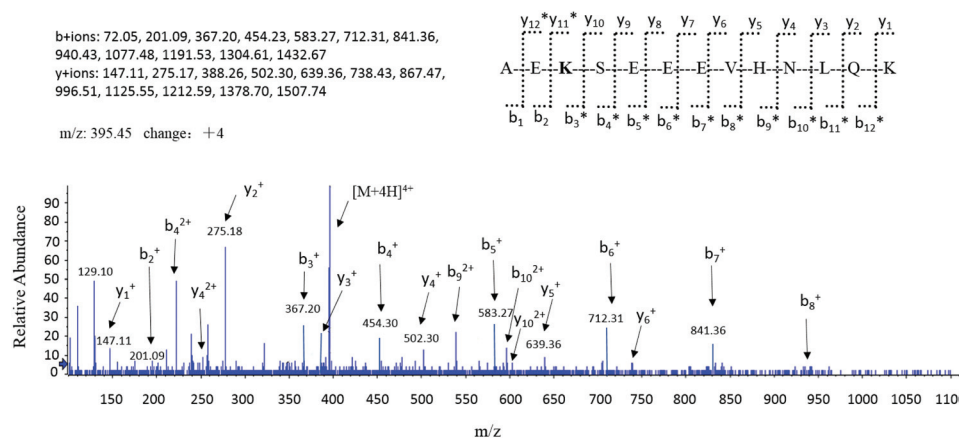
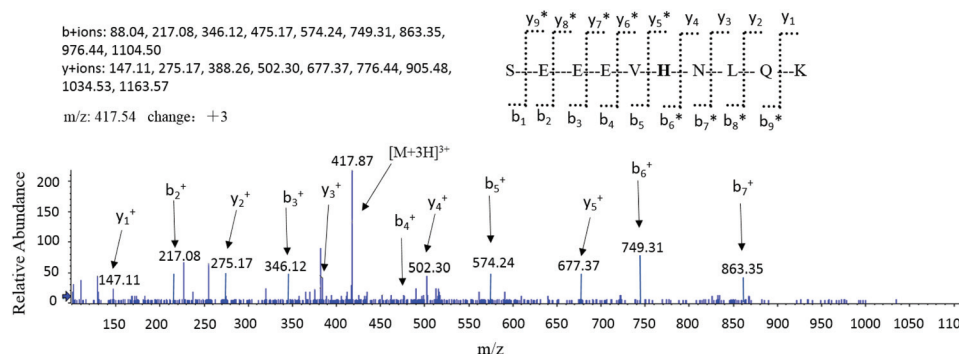
Table 1 List of the acrolein-modified peptides of shrimp tropomyosin identified after date-dependent tandem mass spectrometry acquisition

Acrolein-modified peptide	Modification site(s)	z	m/z	Observed mass	Theoretical mass
AEKSEEEVHNLQK (38)	38	4	395.451	1577.774	1577.769
EEAYKEQIK (38, 38)	221 222	2	607.307	1212.598	1212.603
YKSITDELDTQTFSELSGY (38, 38)	267 268	3	724.674	2170.999	2170.995
SEEEVHNLQK (38)	44	3	417.538	1249.592	1249.594
MDALENQLK (16)	141	2	539.266	1076.517	1076.517
LAMVEADLER (16)	171	2	581.793	1161.571	1161.570
DNAMDRADTLEQQNK (16)	19	3	588.931	1763.772	1763.774

base formation and [O] addition, which were identified by LC-MS-MS.

As shown in Fig. 5–8, the acrolein and [O] adducts of tropomyosin involve two or more nucleophilic sites, as detected by LC-MS-MS analysis. For instance, Fig. 5 shows the acrolein adduct formation on the Lys38 residue in the peptide ³⁶Ala-Glu-Lys-Ser-Glu-Glu-Glu-Val-His-Asn-Leu-Gln-Lys⁴⁸. Fig. 6 shows an example of acrolein adduction in the amino acid side-chains containing histidine⁴⁴ of ³⁹Ser-Glu-Glu-Glu-Val-His-Asn-Leu-Gln-Lys⁴⁸. The formation of acrolein adduct on

the Tyr²²¹ residue was observed in the peptide ²¹⁸Glu-Glu-Ala-Tyr-Lys-Glu-Gln-Ile-Lys²¹⁶ (Fig. 7). Met was modified through [O] addition in the peptide ¹⁶⁹Leu-Ala-Met-Val-Glu-Ala-Asp-Leu-Glu-Arg¹⁷⁸ (Fig. 8). The modified amino acid residues of tropomyosin can alter the allergic epitopes and conformational (secondary or tertiary) structure, which could have an influence on the function and properties. Moreover, based on epitope research, the analysis of the oxidation sites could be of great use to understand the changes of IgE binding capacity during food processing and preservation. Future research

**Fig. 5** MS/MS spectrum for a peptide containing Lys from acrolein-treated shrimp tropomyosin. Acrolein Schiff base adduction (*, +38) was observed on the lysine side-chain group in Ala-Glu-Lys-Ser-Glu-Glu-Glu-Val-His-Asn-Leu-Gln-Lys (acrolein-modified residue in bold type).**Fig. 6** MS/MS spectrum for a peptide containing His from acrolein-treated shrimp tropomyosin. Acrolein Schiff base adduction (*, +38) was observed on the histidine side-chain group in Ser-Glu-Glu-Glu-Val-His-Asn-Leu-Gln-Lys (acrolein-modified residue in bold type).

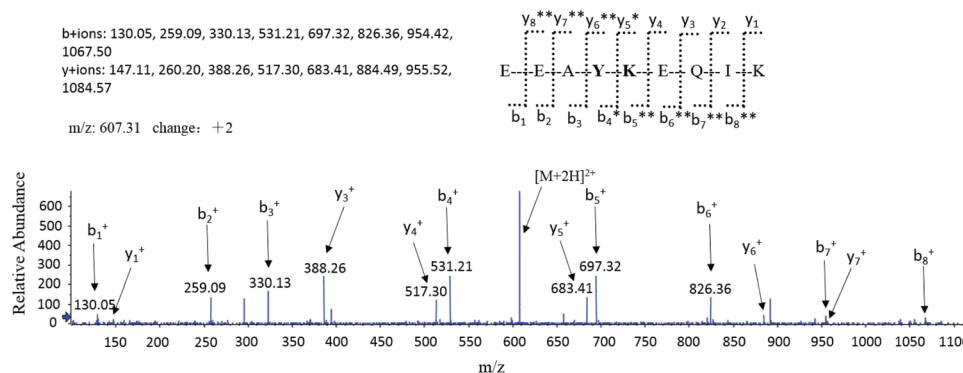


Fig. 7 MS/MS spectrum for a peptide containing Tyr from acrolein-treated shrimp tropomyosin. Acrolein Schiff base adduction (*, +38) was observed on the tyrosine side-chain group in Glu-Glu-Ala-Tyr-Lys-Glu-Gln-Ile-Lys (acrolein-modified residues in bold type).

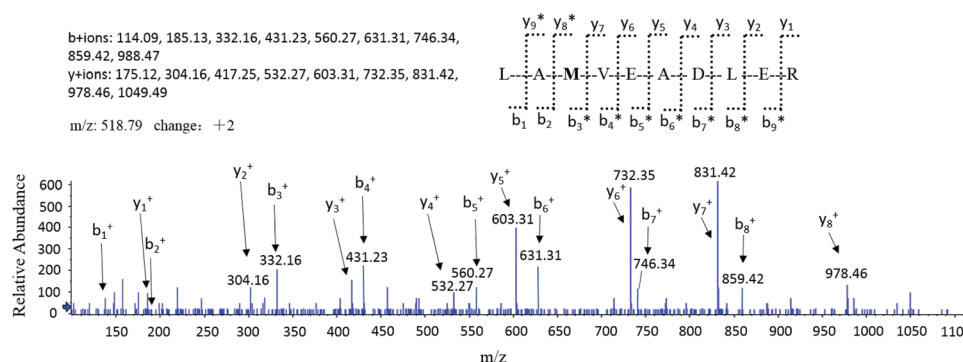


Fig. 8 MS/MS spectrum for a peptide containing Met from acrolein-treated shrimp tropomyosin. [O] adduction (*, +16) was observed on the DL-methionine side-chain group in Leu-Ala-Met-Val-Glu-Ala-Asp-Leu-Glu-Arg ([O]-modified residue in bold type).

should focus on decreasing IgE binding capacity of tropomyosin through elucidating different chemical modifications that occur on account of oxidative damage during processing and preservation.

In conclusion, no matter what modifications induce changes in the secondary and tertiary structure of protein, they could influence the IgE binding capacity. Acrolein-induced modification of amino acid residues in tropomyosin caused conformational structure changes. Therefore, such modification of amino acids in the IgE binding regions could be the main reason for the reduction in IgE binding capacity of shrimp tropomyosin. It is recommended that future research should be done to evaluate the allergenicity *in vivo*. The existing results could provide useful information for modifying IgE binding capacity as happens due to lipid oxidation during shrimp preservation and processing.

Ethical statement

1. All the experiments were performed in compliance with relevant guidelines.

2. All experiments were performed following institutional guidelines.

3. The Food Safety Committee, College of Food Science and Engineering, Ocean University of China has approved all the experiments involved in this particular research work.

4. All animal experiments were performed following established guidelines and the experimental protocol was approved by the Ethical Committee of Experimental Animal Care at Ocean University of China (OUC, P. R. China), no. SPXK20150001.

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