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Transglutaminase-catalysed cross-linking eliminates *Penaeus chinensis* tropomyosin allergenicity by altering protein structure

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ABSTRACT

Transglutaminase (TG) is a major food processing enzyme that induces protein cross-linking. Tropomyosin (TM) is a major shellfish allergen that is responsible for at least 80% of the shrimp allergic incidents. The aim of this research was to investigate the effect of TG on TM allergenicity and reveal the involvement of TM structure alternation. After treated with TG for different time, the allergenicity of TM was assessed by indirect ELISA with shrimp allergic patients' sera, and the structure of TM was characterized by circular dichroism (CD) spectroscopy, ultraviolet (UV) absorption spectroscopy and fluorescence spectroscopy. Results showed that the allergenicity slightly decreased at the early stage of reaction and dramatically decreased at the late stage, which is tightly correlated with the increment of β -turn content and UV absorption. In conclusion, TG modification is an effective and promising food processing method to eliminate TM allergenicity with significant prospect of industrial application.

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KEYWORDS

Tropomyosin; allergenicity; structure; transglutaminase

Introduction

Food allergy is an abnormal immunological response to certain food components, which has emerged as a common public health concern both in developed and developing countries (Nowak-Wegrzyn, 2013; Oyoshi, Oettgen, Chatila, Geha, & Bryce, 2014). With the continuous progress of social progress and globalization, the incidence of allergic diseases has been escalating. About 30% to 40% of people in the world suffer from various allergic diseases, and morbidity and mortality show rising trend year by year (Geha, 2003). At present, there are no effective methods to prevent or eradicate food allergy, commonly used drugs such as antihistamine can only temporarily relieve allergic symptoms (Leurs, Church, & Taglialatela, 2002). Consequently, the only safe and effective way is to avoid eating or

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contacting with allergens nowadays, but most food allergens are the main source of nutrition, and the development of modern food industry makes every food contain many ingredients that do not show the original state, making it difficult to avoid food ingredients completely, so reducing food allergenicity is the first choice to reduce the risk of food allergy.

Shellfish is one of the most frequent causes of food allergy, and it is responsible for the majority of emergency department visits related to severe food allergy (Sicherer, Muñoz-Furlong, & Sampson, 2004). Shellfish allergy is a type I hypersensitivity reaction, which is mediated by the binding of allergens with specific immunoglobulin E (IgE). Tropomyosin (TM) have been identified as the major allergens of shrimp by previous studies (Ayuso, Lehrer, & Reese, 2002; Gonzalez-Fernandez, Alguacil-Guillen, Cuellar, & Daschner, 2018; Motoyama et al., 2008; Shanti, Martin, Nagpal, Metcalfe, & Rao, 1993), which comprises 5–7% of shrimp muscle and is a heat-stable protein with a molecular weight of 36 kDa that can hardly be denatured by common food processing methods (Wang, Li, & Lin, 2008).

During food processing, different methods, such as heating, ultra-high pressure, and enzymatic cross-linking, could influence the allergenicity of allergens through structural changes (Bu, Luo, Zheng, & Zheng, 2009; Jin et al., 2015; Peram, Loveday, Aiqian, & Harjinder, 2013). Enzymatic cross-linking modification of protein method usually refers to the intramolecular or intermolecular cross-linking reaction under the action of enzymes, which can promote the polymerization of the protein. It has been reported that the IgE binding activity of peanut allergen Ara h 1 and 2, and milk allergen casein and lactoprotein were decreased after cross-linked by tyrosinase (Farrell Jr et al., 2004; Laemmli, 1970). The effect of cross-linking by horseradish peroxidase (CHP) or tyrosinase (CTC) on crab TM was also investigated, the result showed that both CTC and CHP reduced the IgE-binding ability (G. Liu et al., 2017).

Transglutaminase (TG) is one of the cross-linking enzymes, which catalyse the junction between amidyl groups such as the epsilon amino acid on polypeptide lysine and the gamma hydroxyl amide group on the glutamic acid, thereby promoting covalent cross-linking between proteins and polypeptides (M. Han, Zhang, Ying, Xu, & Zhou, 2009; Jiang, 2001). This reaction can improve the structure and functional properties of protein and give food protein unique texture and taste, so it has been widely used in meat, aquatic products, soybean products and other food industries. For instance, treating peanut powder containing casein with TG resulted in the polymerization of the main allergen Ara h 2 in peanut and thus decreased the IgE binding activity (Clare, Gharst, Maleki, & Sanders, 2008).

Previous studies have shown 10 linear epitopes in shrimp TM that are responsible for its allergenicity, which contain glutamic acid (E) and lysine (K) abundantly (L. Fu, Wang, Ni, Wang, & Wang, 2018), indicating that these epitopes could be catalysed by TG, which would change the structure of the protein and thus reduce the allergenicity. Consequently, the major goal of the present work was to determine the effect of TG-catalysed cross-linking on the structure and the potential allergenicity of TM in *Penaeus chinensis*.

Material and methods

Human serum

Serum samples were obtained from 20 subjects with confirmed clinical history of allergic responses to *P. chinensis* and five subjects that without allergic response. Shrimp-specific

sera samples were obtained from the Chongqing MaNueAiKe Technology Company and stored at -80°C until use. This procedure as well as the whole study were approved by Zhejiang Gongshang University Ethics Review Committee.

Protein purification

TM was purified from *P. chinensis* according to a previous study (Fu et al., 2017). Shrimp was homogenized with 10 times volume of Buffer A and kept on ice for 20 min, followed by supernatant removal by centrifuging at 8000 rpm for 15 min (4°C). This procedure was repeated for 5 times to maximize the depletion of soluble proteins. After that, insoluble materials were suspended in pre-cooled acetone, stirring 30 min on ice and filtrated by gauze until it becomes white, then the product was placed in ventilator and dried. Then the solid was soaked in Buffer B for 3 days to be dissolved, and the insoluble materials were removed by gauze. After boiling for 20 min and centrifuging at 8000 rpm for 15 min (4°C), ammonium sulphate was added into the supernatant to a final concentration of 30% saturation to perform ammonium sulphate precipitation on ice for 30 min. Finally, the precipitation produced by centrifuged at 8000 rpm for 15 min (4°C) was dissolved in PBS (10 mM, pH 7.4) and stored at -80°C .

Enzymatic cross-linking reaction by TG

The procedure for cross-linking of TM by TG (Yuan Ye Biotechnology, Shanghai, China) was as follows (F. Yuan et al., 2017): TM was diluted with 10 mM PBS (pH 7.4) and TG was added to indicate enzyme to substrate ratio. The reaction volume was 100 μL and the final concentration of substrate was 0.1 $\mu\text{g}/\mu\text{L}$. The reactions were performed in a shaker at 37°C for indicated times. After the end of the reaction, the samples were heated at 90°C for 10 min to inactivate enzymes.

Electrophoretic and ELISA analysis

The reaction samples were collected and detected by 12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) coupled with Coomassie brilliant blue staining. The relative protein concentrations were measured by Image J 180. The IgE-binding activity of samples was measured by ELISA: each sample was coated on a 96-well plate in 0.05 M carbonate buffer overnight at 4°C . After washing, blocking with 3% bovine serum albumin (in 0.01 M pH 7.4 PBS) for 2 h at 37°C , and washing again, the plate was incubated with serum samples (diluted 1:200) for 1.5 h at 37°C . After washing, the plates were incubated with HRP goat anti-human IgE serum (diluted 1:2500, Novus Biologicals, USA) for 1.5 h at 37°C , and washed again. Finally, the plate was incubated with TMB at 37°C for 15 min, the reaction was terminated by 2 M H_2SO_4 , and the absorbance was measured at 450 nm by Versa max Microplate reader from Molecular Devices (Sunnyvale, USA). Meanwhile, untreated TM was taken as the positive control, and untreated TM with non-allergic patient's sera severed as the negative control. The allergenicity reduction rate was calculated as the following formula:

Allergenicity reduction rate = (control OD450 – experiment OD450) / experiment OD450 $\times 100\%$.

Circular dichroism analysis

CD spectroscopy was performed by a circular dichroism spectrophotometer (JASCO, Japan) in the spectral range from 190 nm to 260 nm. Protein concentration was adjusted to 0.1 mg/mL for analysis. A scan speed at 50 nm per min and a bandwidth of 1 nm were used. The contents of different secondary structures were calculated with JASCO secondary structure software (JASCO, Japan) based on the spectrum results.

Fluorescence spectroscopy analysis

Fluorescence spectroscopy was performed by a fluorescence spectrophotometer (F-7000 FL, Mei Pu Da, Shang Hai) in the spectral range from 295 nm to 450 nm at room temperature. 1.5 mL protein sample with the concentration of 0.1 mg/mL was used for analysis. The maximum excitation wavelength is 280 nm and the scanning speed is 240 nm/min. The slits of the excitation and emission paths are 10 and 5 nm, respectively.

UV absorption spectroscopy analysis

The ultraviolet absorption spectra of the samples at 200–800 nm were scanned at room temperature with a scanning density of 1 nm by a spectrophotometer (Hitachi Limited, Japan). Protein concentration was adjusted to 0.1 mg/mL for analysis. Each sample is scanned for three times to get the average value.

Statistical analysis

Each experiment was repeated at least three times according to a completely randomized design. Statistical differences were analysed using Student's *t* test by GraphPad Prism 7.0 (GraphPad Software, USA), and the correlations were analysed using Pearson bivariate correlation statistics by SPSS 17.0 (IBM, USA). Data were presented as mean $\pm$ SD and analysed by ANOVA and Dunn's test, the difference was considered statistically significant when $p < 0.05$.

Results

TM cross-linking catalysed by TG

In order to destruct shrimp TM, we tried to treat TM with the cross-linking enzyme TG, and assess the effect on protein structure by SDS-PAGE. As shown in [Figure 1](#), the main TM band (37 kDa) became shallow after enzymatic treatment for 8 h, and the signal in high-molecular-weight region (about 200 and 76 kDa) was increased gradually, which indicated the formed polymers after cross-linking. In addition, the signal of polymers strengthened with the increase of enzyme amount, this dose-dependent manner further confirmed the specificity of TG enzymatic activity.

Then we evaluated the allergenicity of TM by determining the IgE-binding capacity using ELISA with allergic patients' sera. However, the allergenicity of TM only slightly decreased after TG treatment, and did not correlated with either the dosage of enzyme or the amount of intact TM monomer ([Figure 2](#)). We concluded that the cross-linking

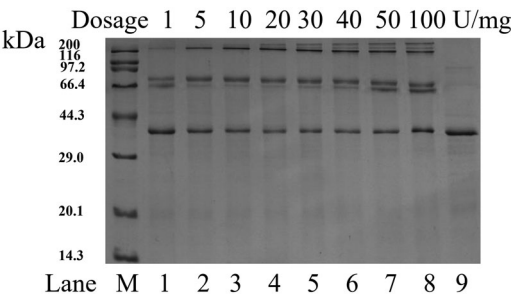


Figure 1 . SDS-PAGE analysis of TM treated with different dose of TG. Purified TM was incubated with different concentration of TG (1, 5, 10, 20, 30, 40, 50, 100 U/mg mass ratio of enzyme to substrate) at 37° C for 8 h. The reaction products were separated by SDS-PAGE and detected by Coomassie brilliant blue staining. M, protein marker; lane 9, untreated TM.

by TG treatment did not directly affect the IgE epitopes of TM, and the TM polymer showed comparable allergenicity with the monomer.

The enzyme kinetics of TG on modifying TM

Since the treatment with TG for a fixed period of time only showed the marginal effect on TM allergenicity, we then treated TM with a particular dose of TG (1 U/mg) for different time period to further investigate the enzymatic kinetics. As shown in [Figure 3](#), TM polymer appeared at the beginning of reaction (0.5 h) and increased with the reaction prolonging, the reaction terminated at 24 h, at which time point almost all the TM were cross-linked and the TM monomer was undetectable. It should be noted that the signal of polymer was also decreased after 16 h, which may due to the dramatic structural change or the formation of extremely large-molecular-weight polymers that cannot enter the polyacrylamide gel or even turn into precipitate. These results indicated that the cross-linking of TM by TG is a relatively slow and multi-step process.

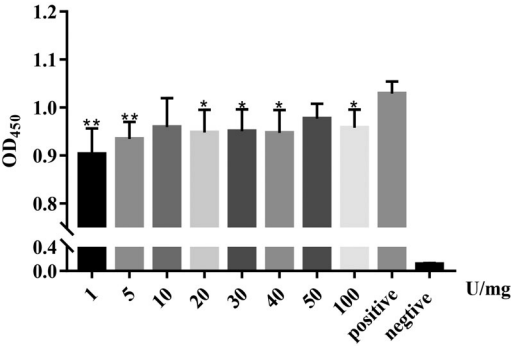


Figure 2. Allergenicity change of TM treated with different dose of TG. Purified TM was incubated with different concentration of TG, and the allergenicity of the products were measured by indirect ELISA using sera from shrimp allergic patients, results are expressed as the optical density at 450 nm. The untreated TM was used as positive control and non-allergic patient's sera were used in the negative control. Data are represented as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$ compared with positive control.

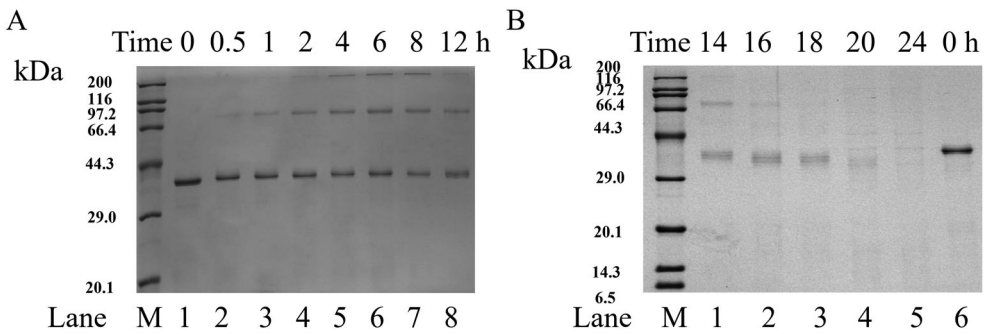


Figure 3. SDS-PAGE analysis of TM treated with TG for different time. Purified TM was incubated with TG (1 U/mg) at 37°C for indicated time (0.5, 1, 2, 4, 6, 8, 12 h in (A) and 14, 16, 18, 20, 24 h in (B)). M, protein marker.

To determine if the extension of reaction time can affect the allergenicity, the IgE-binding capacity of reaction products was assessed by ELISA (Figure 4). The results showed that the allergenicity was barely changed at the first 12 h, however, it started to decrease after 14 h, dramatically decreased with longer time, and almost all the allergenicity was eliminated at 24 h. Interestingly, the allergenicity is tightly correlated with the amount of the overall TM signal (both monomer and polymer) on SDS-PAGE (Figure 3), which had a well linear correlation with the R of 0.89 (Table 1), suggesting that only the intact or mild-modified soluble TM can be recognized by IgE and thus trigger downstream allergenic responses.

Structural change of TM during TG modification

To investigate the mechanisms of TG-induced TM allergenicity elimination, we further determined the structural changes of TM throughout the reaction process. CD

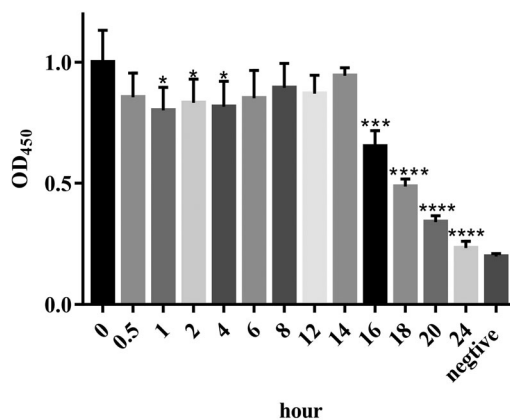


Figure 4. Allergenicity change of TM treated with TG for different time. Purified TM was incubated with TG for different time, and the allergenicity of the products were measured by indirect ELISA using sera from shrimp allergic patients, results are expressed as the optical density at 450 nm. The untreated TM was used as positive control and non-allergic patient's sera were used in the negative control. Data are represented as mean $\pm$ SD. * $P < 0.05$, **** $P < 0.0001$ compared with positive control.

Table 1. Pearson bivariate correlation analysis of allergenicity reduction rate with other parameters.

	Pearson Correlation	Significance (2-tailed)
Reduction rate	1	–
Intensity	.704**	0.007
Absorbance	.878**	0
α-Helix	–.867**	0
β-Sheet	.634*	0.02
β-Turn	.920**	0
Random coil	.641*	0.018
SDS-PAGE signal	–.890**	0

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

spectroscopy was first used to characterize the secondary structure of TM (Figure 5). Intact TM showed two negative peaks at 208 and 220 nm, indicating that the dominant secondary structure of TM is an α-helix. After TG treatment, these two peaks decreased in a time-dependent manner, and the curve dramatically changed after 24 h treatment. Further analyses performed with Yang software showed that TG treatment decreased the content of α-helix, and increased β-turn and random coil. After 24 h treatment, the α-helix decreased to 29.6%, β-turn, random coil and β-sheet increased to 38.4%, 31.4%, and 0.7%, respectively (Figure 6).

In addition to the CD spectra, intrinsic fluorescence spectra and UV absorption spectra were also determined to characterize the structural change during TG modification. TM exhibited a fluorescence emission maximum at 341 nm and the value shows a blue shift for 1–5 nm after TG treatment, indicating that the microenvironment of tryptophan residues was changed throughout the reaction (Figure 7). What’s more, the emission intensity dramatically increased after more than 20 h treatment, indicating that protein structure altered from folding to stretching, and thus exposed more hydrophobic groups (J. Han, Chen, Wu, Huang, & Ge, 2017).

On the other hand, the UV absorption peak of TM appeared at about 280 nm and showed a blue-shift with the extension of reaction time, meanwhile the overall absorbance

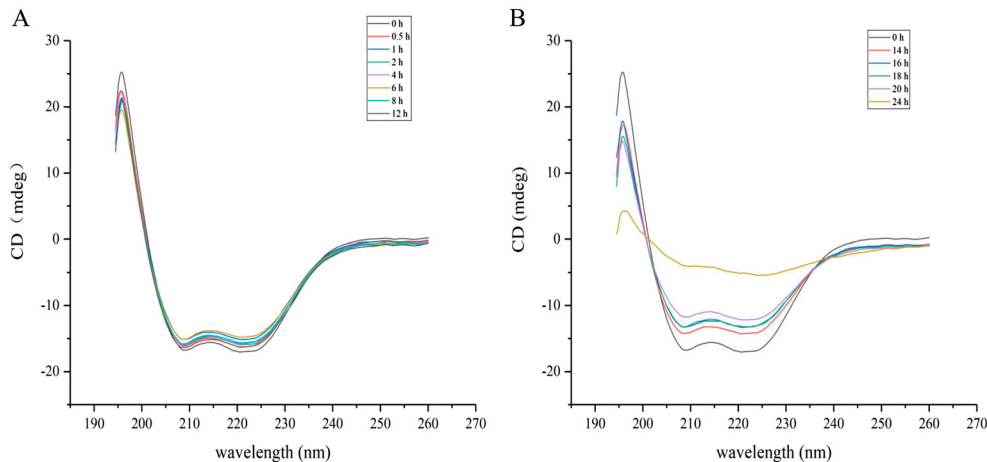


Figure 5. CD spectroscopy analysis of the TG-treated TM. TG treated TM samples were adjusted to 0.1 mg/mL and the CD spectrum was measured.

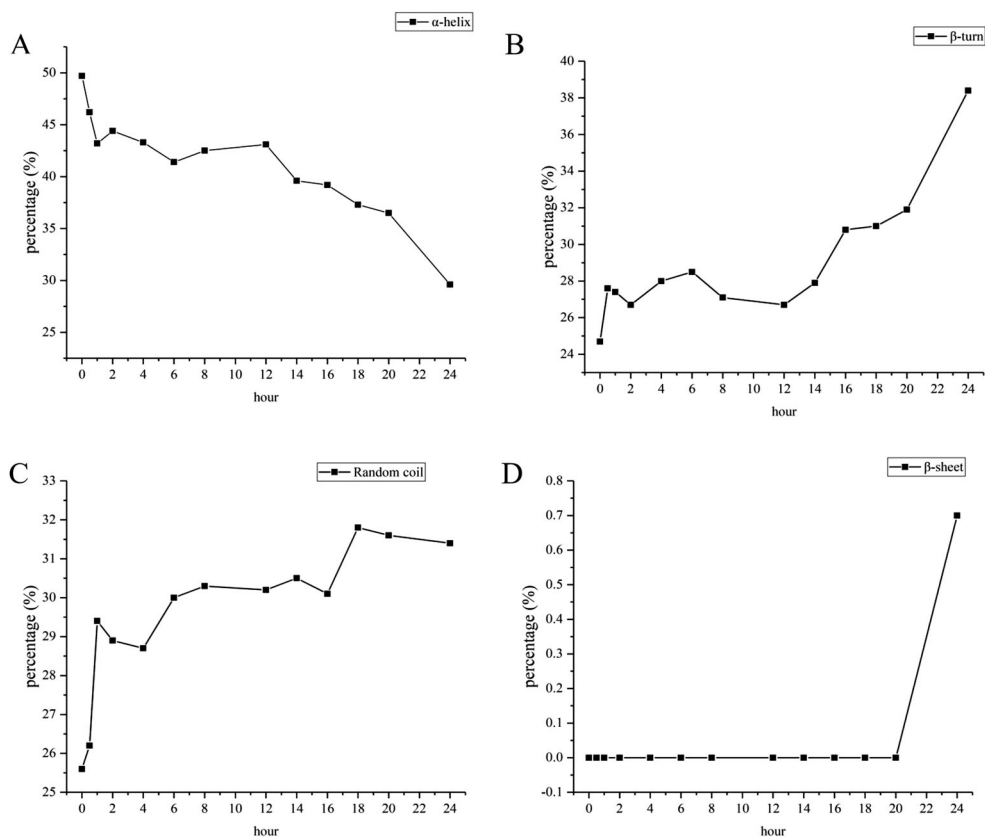


Figure 6. The secondary structure content of the TG-treated TM. The secondary structure content of TG-treated TM sample was calculated by JASCO secondary structure software based on the spectrum results. (A) α -helix content. (B) β -turn content. (C) Random coil content. (D) β -sheet content.

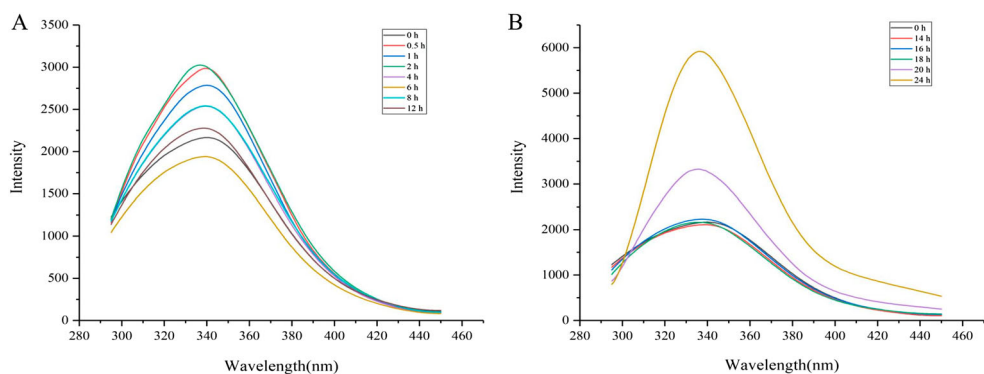


Figure 7. Fluorescence spectroscopy analysis of TG-treated TM. TG treated TM samples were adjusted to 0.1 mg/mL and the fluorescence intensity spectrum was measured.

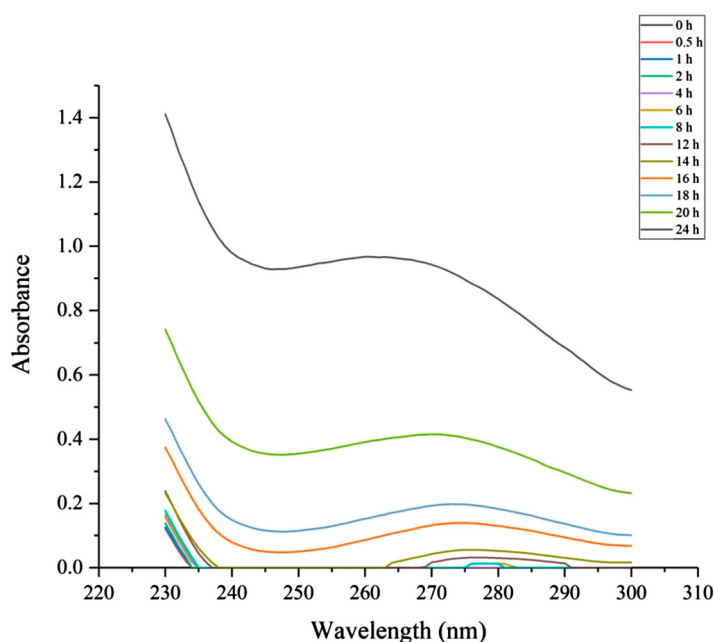


Figure 8. UV absorption spectroscopy analysis of TG-treated TM. TG treated TM samples were adjusted to 0.1 mg/mL and the UV absorption spectrum was measured.

was also significantly increased (Tong et al., 2012), indicating that specific amino acids such as tryptophan, tyrosine and phenylalanine were exposed following TG treatment (Figure 8).

Overall, these results demonstrated that TG treatment resulted in the structural change of TM, which should be involved in the change of TM allergenicity.

TG-induced TM structural change is correlated with allergenicity elimination

To investigate the contribution of each type of structure (α -helix, β -sheet, β -turn and random coil) on allergenicity, we compared the change of each structure type with allergenicity reduction rate under TG treatment (Figure 9(A)). Compared with β -sheet and random coil, which were not significantly changed after TG treatment, α -helix was consistently reduced with the elongation of treatment, and β -turn was dramatically increased after long-time (12 h) reaction. Similarly, the allergenicity was also reduced after 12 h, suggesting that β -turn could be the predominant contributor of TM allergenicity. More exact relationship between TG-induced TM structural change and allergenicity elimination was presented as the statistical correlation calculated by Pearson bivariate correlation statistics. As shown in Table 1, the Pearson correlation (R) between allergenicity reduction rate and the change of α -helix, β -sheet, β -turn and random coil was 0.867, 0.634, 0.92 and 0.641, demonstrating that TM allergenicity was most tightly correlated with β -turn, followed by α -helix.

In addition, we also assessed the relationship between allergenicity and fluorescence intensity (Figure 9(B)) or UV absorbance (Figure 9(C)) of TM, and calculated the precious

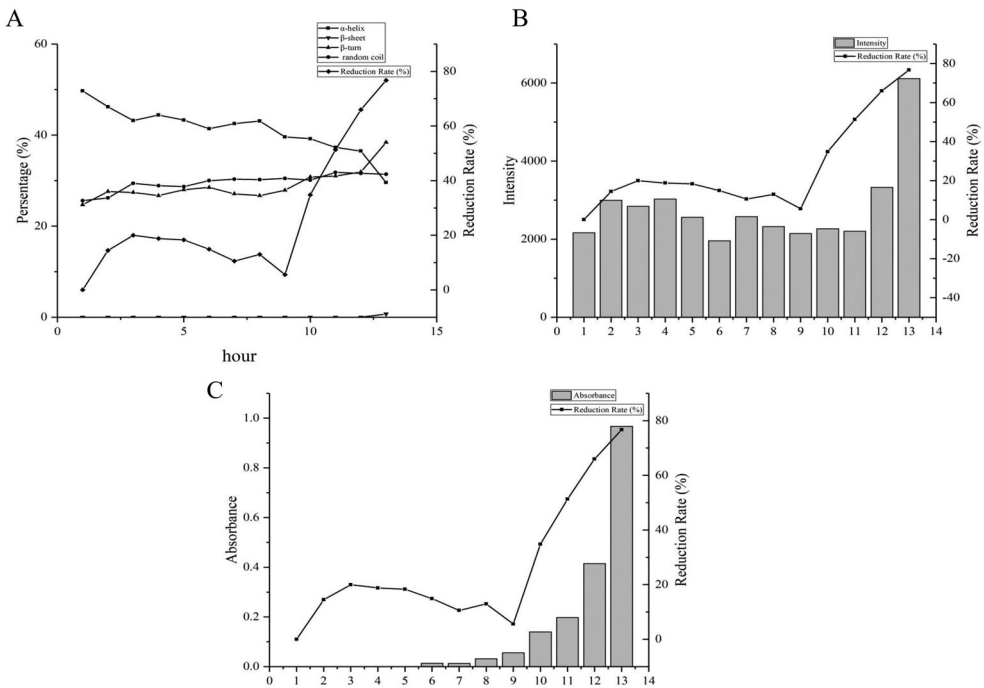


Figure 9. Relationship between TM structural change and allergenicity reduction rate of TG-treated TM. (A) The calculated secondary structure content and the allergenicity reduction rate of TG-treated TM were presented. (B) The fluorescence intensity and the allergenicity reduction rate of TG-treated TM were presented. (C) The UV absorbance and the allergenicity reduction rate of TG-treated TM were presented.

statistical correlations (Table 1). The results suggested that UV absorbance ($R = 0.878$) can partially reflect the allergenicity change better than the fluorescence intensity ($R = 0.704$).

Discussion

A large number of literatures have proved that enzymatic cross-linking can reduce the binding activity of IgE to allergens. For example, cross-linking with laccase can effectively reduce the allergenicity of casein in milk (Tantoush et al., 2011); similarly, cross-linking with tyrosinase and caffeic acid decreased the binding activity of peanut allergen (Chung, Kato, & Champagne, 2010; Fei et al., 2016); furthermore, transglutaminase treatment can significantly reduce the allergenicity of milk and wheat allergens (Łacka & Bryzewska, 2006; F. Z. Yuan et al., 2018). In the present study, we also demonstrated that the potential allergenicity of TM can be reduced by TG-induced cross-linking. Interestingly, our results showed that TM allergenicity only marginally decreased at the early stage of reaction and dramatically decreased after a long time reaction, suggesting that TG-mediated TM cross-linking is a relatively slow and multi-step process. Moreover, the SDS-PAGE results also confirmed the suggestion, while TM only polymerized at the early stage of reaction, it turned into large-molecular-weight polymers and cannot be detected. The high correlation between SDS-PAGE signal and allergenicity further

implied that the products of late-stage reaction lost the IgE binding ability and thus become not allergenic. Previous studies have demonstrated the effectiveness of several methods, such as power ultrasound (X. Y. Li, Li, Lin, & Samee, 2011), pH shifts (Lin et al., 2015), and malonaldehyde oxidization (Lv et al., 2014; Song et al., 2015) in reducing TM allergenicity. Whereas the present study provides another approach to achieve this purpose, which is efficient, mild, and does not require complicated equipment, and therefore is a promising method to be applied in the industry.

To investigate the detailed structural changes of TM after TG treatment, we determined the protein structure by CD spectra, intrinsic fluorescence spectra and UV absorption spectra. Based on the CD data, the content of each type of secondary structures were calculated. The results showed that the content of α -helix was gradually decreased at a roughly uniform speed throughout the process and random coil was increased in a similar way, implying that α -helix is deconstructed into random coil by TG-induced cross-linking, which is consistent with the conclusion of a previous study that TG enzyme-catalysed cross-linking would cause uncoiling of the alpha-helix of myofibrillar protein and destroy the stability of its secondary structure (C. Li, Xiong, & Chen, 2012). On the contrary, the percentage of β -turn is significantly increased only at the late stage of reaction, which is quite similar to the change of allergenicity. Further statistical analysis also proved the tight correlation between TM allergenicity and β -turn, implying that the increment of β -turn could be a major reason for allergenicity decrement.

In addition to secondary structure, more structural properties were determined by fluorescence spectroscopy and UV absorption spectroscopy. The alternation of the spectra indicated the expose of inner-molecular amino acids and the change of hydrophobic microenvironment, which is due to the crossing-linking of surface amino acids including glutamic acid and lysine (J. Liu, He, Tang, & Chen, 2008), and reflects the conformational change of TM during TG treatment. While the correlation between fluorescence spectra and allergenicity is lower, the UV absorbance was tightly negatively correlated with allergenicity, indicating that the expose of inner amino acids especially tryptophan, tyrosine and phenylalanine can disrupt the IgE epitopes of TM. Moreover, the linear regression analysis provided a potential way to rapid test the efficiency of allergenicity elimination based on simple UV absorption spectroscopy.

In conclusion, this work showed that TG-catalysed cross-linking could induce structural changes in TM, which could be a relatively slow and multi-step process, resulting in the reduction of IgE binding capacity. Specifically, the formation of extra β -turn and the expose of inner tryptophan, tyrosine and phenylalanine could be the predominant conformational changes responsible for allergenicity reduction. Compared with other methods such as heating and ultra-high pressure, TG-catalysed cross-linking showed high efficiency under mild conditions and the effect can be easily predicted by UV absorption spectroscopy, therefore it is more suitable to be used in food processing. However, the requirement of long time reaction is a disadvantage of the method and should be improved in future studies.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

- Ayuso, R., Lehrer, S. B., & Reese, G. (2002). Identification of continuous, allergenic regions of the major shrimp allergen Pen a 1 (tropomyosin). *International Archives of Allergy and Immunology*, 127(1), 27–37.
- Bu, G., Luo, Y., Zheng, Z., & Zheng, H. (2009). Effect of heat treatment on the antigenicity of bovine γ -lactalbumin and γ -lactoglobulin in whey protein isolate. *Food & Agricultural Immunology*, 20(3), 195–206.
- Chung, S. Y., Kato, Y., & Champagne, E. T. (2010). Polyphenol oxidase/caffeic acid may reduce the allergenic properties of peanut allergens. *Journal of the Science of Food & Agriculture*, 85(15), 2631–2637.
- Clare, D. A., Gharst, G., Maleki, S. J., & Sanders, T. H. (2008). Effects of transglutaminase catalysis on the functional and immunoglobulin binding properties of peanut Flour Dispersions containing casein. *Journal of Agricultural & Food Chemistry*, 56(22), 10913.
- Farrell Jr, H. M., Jimenezflores, R., Bleck, G. T., Brown, E. M., Butler, J. E., Creamer, L. K., ... Swaisgood, H. E. (2004). Nomenclature of the proteins of cows' milk—sixth revision. *Journal of Dairy Science*, 87(6), 1641–1674.
- Fei, D. X., Liu, Q. M., Chen, F., Yang, Y., Chen, Z. W., Cao, M. J., & Liu, G. M. (2016). Assessment of the sensitizing capacity and allergenicity of enzymatic cross-linked arginine kinase, the crab allergen. *Molecular Nutrition & Food Research*, 60(7), 1707–1718.
- Fu, L., Fu, S., Wang, Y., Wang, C., Zhang, Y., Wu, Q., & A, M. A. (2017). Optimization of separation and purification of myosin sulfate from *Penaeus vannamei*. *Food Science*, 38(18), 187–192.
- Fu, L., Wang, J., Ni, S., Wang, C., & Wang, Y. (2018). Identification of allergenic epitopes and critical amino acids of major allergens in Chinese shrimp (*Penaeus chinensis*) by Immunoinformatics coupled with Competitive-binding Strategy. *Journal of Agricultural & Food Chemistry*, 66(11), 2944–2953.
- Geha, R. S. (2003). Allergy and hypersensitivity: Nature versus nurture in allergy and hypersensitivity. *Current Opinion in Immunology*, 15(6), 603–608.
- Gonzalez-Fernandez, J., Alguacil-Guillen, M., Cuellar, C., & Daschner, A. (2018). Possible allergenic role of tropomyosin in patients with adverse reactions after fish intake. *Immunological Investigations*, 47(4), 416–429. doi:10.1080/08820139.2018.1451882
- Han, J., Chen, Y., Wu, Y., Huang, W., & Ge, Y. (2017). The effect of ultrahigh pressure on the antigenicity and structure of the myosin of prawns by the combination of temperature and pressure. *Spectroscopy and Spectral Analysis*, 37(2), 522–526.
- Han, M., Zhang, Y., Ying, F., Xu, X., & Zhou, G. (2009). Effect of microbial transglutaminase on NMR relaxometry and microstructure of pork myofibrillar protein gel. *European Food Research & Technology*, 228(4), 665–670.
- Jiang, B. (2001). Properties of glutamine transaminase and its application in food. *Chinese Food Additives*, 4, 34–36.
- Jin, Y., Deng, Y., Qian, B., Zhang, Y., Liu, Z., & Zhao, Y. (2015). Allergenic response to squid (*Todarodes pacificus*) tropomyosin Tod p1 structure modifications induced by high hydrostatic pressure. *Food & Chemical Toxicology*, 76, 86–93.
- Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. *Nature*, 227(5259), 680–685.
- Łacka, A., & Bryszewska, M. (2006). The use of transglutaminase in the reduction of immunoreactivity of wheat flour AU - Leszczyńska, Joanna. *Food and Agricultural Immunology*, 17(2), 105–113. doi:10.1080/095401006000870279

- Leurs, R., Church, M. K., & Taglialatela, M. (2002). H1-antihistamines: Inverse agonism, anti-inflammatory actions and cardiac effects. *Clinical Experimental Allergy*, 32(4), 489–498.
- Li, X. Y., Li, Z. X., Lin, H., & Samee, H. (2011). Effect of power ultrasound on the Immunoactivity and texture changes of shrimp (*Penaeus vannamei*). *Czech Journal of Food Sciences*, 29(5), 508–514. doi:10.17221/242/2009-cjfs
- Li, C., Xiong, Y. L., & Chen, J. (2012). Oxidation-induced unfolding facilitates myosin cross-linking in myofibrillar protein by microbial transglutaminase. *Journal of Agricultural & Food Chemistry*, 60(32), 8020.
- Lin, H. X., Li, Z. X., Lin, H., Song, Y. N., Lv, L. T., & Hao, Z. N. (2015). Effect of pH shifts on IgE-binding capacity and conformational structure of tropomyosin from short-neck clam (*Ruditapes philippinarum*). *Food Chemistry*, 188, 248–255. doi:10.1016/j.foodchem.2015.05.007
- Liu, J., He, Z., Tang, X., & Chen, J. (2008). Effect of alcalase hydrolysis followed by transglutaminase treatment on the foaming properties of soybean protein isolate (SPI). *Journal of Biotechnology*, 136(4), S730–S730.
- Liu, G., Hu, M., Sun, L. C., Han, X., Liu, Q., Alcocer, M., ... Liu, G. M. (2017). Allergenicity and oral tolerance of enzymatic cross-linked tropomyosin evaluated using cell and mouse models. *Journal of Agricultural & Food Chemistry*, 65(10), 2205–2213. doi:10.1021/acs.jafc.6b05816
- Lv, L. T., Lin, H., Li, Z. X., Song, Y. N., Lin, H. X., & Wang, A. Y. (2014). Identification of oxidative modification of shrimp (*Metapenaeus ensis*) tropomyosin induced by malonaldehyde. *European Food Research and Technology*, 239(5), 847–855. doi:10.1007/s00217-014-2281-1
- Motoyama, K., Suma, Y., Ishizaki, S., Nagashima, Y., Lu, Y., Ushio, H., & Shiomi, K. (2008). Identification of tropomyosins as major allergens in Antarctic krill and Mantis shrimp and their amino acid sequence characteristics. *Marine Biotechnology*, 10(6), 709–718.
- Nowak-Węgrzyn, A. (2013). Future therapies for food allergy. *Przegląd Lekarski*, 70(12), 1065–1070.
- Oyoshi, M. K., Oettgen, H. C., Chatila, T. A., Geha, R. S., & Bryce, P. J. (2014). Food allergy: Insights into etiology, prevention and treatment provided by murine models. *Journal of Allergy & Clinical Immunology*, 133(2), 309–317.
- Peram, M. R., Loveday, S. M., Aiqian, Y., & Harjinder, S. (2013). In vitro gastric digestion of heat-induced aggregates of β -lactoglobulin. *Journal of Dairy Science*, 96(1), 63–74.
- Shanti, K. N., Martin, B. M., Nagpal, S., Metcalfe, D. D., & Rao, P. V. (1993). Identification of tropomyosin as the major shrimp allergen and characterization of its IgE-binding epitopes. *Journal of Immunology*, 151(10), 5354.
- Sicherer, S. H., Muñoz-Furlong, A., & Sampson, H. A. (2004). Prevalence of seafood allergy in the United States determined by a random telephone survey. *Journal of Allergy & Clinical Immunology*, 114(1), 159–165.
- Song, Y. N., Li, Z. X., Lin, H., Du, S. Y., Hao, Z. N., Lin, H. X., & Zhu, Z. (2015). Effect of malondialdehyde treatment on the IgE binding capacity and conformational structure of shrimp tropomyosin. *Food Chemistry*, 175, 374–380. doi:10.1016/j.foodchem.2014.12.001
- Tantoush, Z., Stanic, D., Stojadinovic, M., Ognjenovic, J., Mihajlovic, L., Atanaskovic-Markovic, M., & Velickovic, T. C. (2011). Digestibility and allergenicity of β -lactoglobulin following laccase-mediated cross-linking in the presence of sour cherry phenolics. *Food Chemistry*, 125(1), 84–91.
- Tong, P., Gao, J., Chenab, H., Zhang, Y., Jian, S., Wichers, H., ... Liu, F. (2012). Effect of heat treatment on the potential allergenicity and conformational structure of egg allergen ovotransferrin. *Food Chemistry*, 131(2), 603–610.
- Wang, X., Li, Z., & Lin, H. (2008). Identification and physicochemical properties of major allergens of *Penaeus chinensis*. *Journal of Fisheries of China*, 32(2), 273–278.
- Yuan, F. Z., Ahmed, I., Lv, L. T., Li, Z. J., Li, Z. X., Lin, H., ... Ma, J. J. (2018). Impacts of glycation and transglutaminase-catalyzed glycosylation with glucosamine on the conformational structure and allergenicity of bovine beta-lactoglobulin. *Food & Function*, 9(7), 3944–3955. doi:10.1039/c8fo00909k
- Yuan, F., Lv, L., Li, Z., Mi, N., Chen, H., & Lin, H. (2017). Effect of transglutaminase-catalyzed glycosylation on the allergenicity and conformational structure of shrimp (*Metapenaeus ensis*) tropomyosin. *Food Chemistry*, 219, 215–222. doi:10.1016/j.foodchem.2016.09.139