



Changes in structure and allergenicity of shrimp tropomyosin by dietary polyphenols treatment

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ABSTRACT

Here, the potential allergenicity of shrimp tropomyosin (TM) after conjugation with chlorogenic acid (CA) and (–)-epigallo-catechin 3-gallate (EGCG) was assessed. Conformational structures of TM-polyphenol complexes were detected using SDS-PAGE, circular dichroism (CD), and fluorescence. Potential allergenicity was assessed by immunological methods, a rat basophil leukemia cell model (RBL-2H3), and *in vivo* assays. Indirect ELISA showed that TM-polyphenol complexes caused a conformational change to TM structure, with decreased IgG/IgE binding capacity significantly fewer inflammatory mediators were released with EGCG-TM and CA-TM in a mediator-releasing RBL-2H3 cell line. Mice model showed low allergenicity to serum levels of TM-specific antibody and T-cell cytokine production. EGCG-TM and CA-TM might reduce the potential allergenicity of shrimp TM, which could be used to produce hypoallergenic food in the food industry.

1. Introduction

Shrimp is a source of dietary protein that is widely consumed globally, especially in China, Japan and North America. However, as one of the major allergens among the “big eight” foods (Rona et al., 2007), the increased consumption of shrimp products is causing adverse reactions, such as urticaria, asthma and life-threatening anaphylactic shock (Lee, Gerez, Shek, & Lee, 2012; Lopata, O’Hehir, & Lehrer, 2010).

Tropomyosin (TM) is the major shrimp allergen. It is a ~36-kDa myofibrillar protein (Ayuso, Lehrer, & Reese, 2002) that could cause different allergic reactions to shrimp (Srinroch, Srisomsap, Chokchai-chamnankit, Punyarit, & Phiriyangkul, 2015; Gámez et al., 2014). So far, most research on allergen proteins has focused on physical and chemical technologies (Jambrak, Mason, Lelas, Paniwnyk, & Herceg, 2014; Xu, Wang, & Xiang, 2015). Processing methods used to reduce or eliminate the allergenicity during food processing include ultrasonography (Li, Yu, Ahmedna, & Goktepe, 2013), high pressure (Li, Zhu, Zhou, & Peng, 2012) and heat (Kamath, Rahman, Komoda, & Lopata, 2013). Out of potential methods to alter allergens, polyphenols could be used, as they interact with allergen proteins, changing structural and immunologic characteristics (Singh, Holvoet, & Mercenier, 2011). These methods could impact the allergenicity of allergen proteins by changing

their structure; thus, modifying allergen epitopes.

Polyphenols bind proteins via non-covalent and covalent interactions (Ozdam, Capanoglu, & Altay, 2013). Tea and Coffee are polyphenol-rich foods that provide an important source of polyphenols in the diet (Kilmartin & Hsu, 2003). The main active polyphenols of coffee beans and green tea are chlorogenic acid (CA) and (–)-epigallo-catechin 3-gallate (EGCG), respectively (Tajik, Tajik Mack & Enck., 2017; Singh, Sankar & Srivastave, 2011). In food processing, the properties of proteins might be severely affected after conjugation with polyphenols (Rohn, 2014; Feng, Cai, Wang, Li, & Liu, 2018; Yin, Yang, Zhao, & Li, 2014; Liu, Ying, Cai, & Le, 2017; Liu, Ma, McClements, & Gao, 2017). The potential allergenicity of β -lactoglobulin and ovalbumin might also be impacted by covalent interactions with dietary polyphenols (Wu et al., 2018; He et al., 2019). However, knowledge on the structural changes and allergenicity of shrimp TM *in vitro* and *in vivo* after conjugation with polyphenol is lacking, despite it representing a potential approach for developing hypoallergenic product development.

Here, we prepared TM-polyphenol complexes (EGCG-TM and CA-TM) using radical method to ascertain whether the conformational structural changes to EGCG-TM and CA-TM could influence the allergenicity of TM. Structural modifications were detected using SDS-PAGE, CD and fluorescence. The IgG/IgE binding capacity of TM was evaluated

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using indirect ELISA. Potential allergenicity was assessed using RBL-2H3 cell and in vivo assays. Our results are expected to provide pre-clinical data for developing innovative hypoallergenic food products.

2. Materials and methods

2.1. Samples and chemicals

EGCG, CA, and 1-anilino-8-naphthalene-sulfonate (ANS) were obtained from Sigma (St. Louis, MO, USA). The precision marker was purchased from Fisher Scientific (Fair Lawn, NJ, USA). HRP-labelled goat anti-rabbit IgG and anti-human IgE were purchased from Zhongshan Jinqiao Co. (Beijing, China). ELISA kits of histamine, prostaglandin D2, cysteinyl leukotriene, mouse mast-cell protease-1 (mMCP-1), IL-4, IL-13, and interferon (IFN)- γ were obtained from R&D systems (Minneapolis, MN, USA).

2.2. Human sera

Human sera from eight patients allergic to shrimp were supplied by Qingdao Eighth People's Hospital, China (medical ethics committee number: QD-EPH-20191018545, Qingdao, China). Specific IgE levels were evaluated by the ImmunoCAP system (Phadia AB, Uppsala, Sweden). An allergic serum with Specific IgE > 0.35 (KU/L) was defined as being effective (Table 1, supplementary material). After sera were obtained, they were mixed at the same volume, and the pool of sera was used for the following assays.

2.3. Protein purification and preparation of EGCG-TM and CA-TM

Shrimp TM was obtained in the laboratory following the method of Huang and Ochiai (2005). The free radical method is widely used for preparing protein-polyphenol covalent conjugates. TM-polyphenol complexes were prepared following Wu et al. (2018). In briefly, purified TM (8 mg mL⁻¹) was dissolved in 100 mL distilled H₂O, then 5.0 mol/L H₂O₂ (1 mL) and 0.25 g ascorbic acid were added at 25 °C for 2 h under atmospheric air conditions. The solutions were incubated with 0.6 mM EGCG and CA for 48 h at 4 °C. After the reaction, free EGCG and CA were removed from the solution by dialysis for 48 h at 4 °C. Untreated TM was used as a control. The contents of phenolic conjugates were detected by the Foline-Ciocalteu method (Xu et al., 2019), using EGCG and CA as separate standards.

2.4. Protein SDS-PAGE

Protein SDS-PAGE was using following the protocols of Laemmli (1970) with a few modifications. Sample solutions were combined with loading buffer (4:1) and were heated for 7 min at 100 °C. Then SDS-PAGE was carried out with 12% prefabricated gel at 120 V. After running it, the gel was stained with Coomassie Brilliant Blue R-250, and was then de-stained with deionized water. SDS-PAGE results were detected by a ChemiDoc XRS Imaging System and Image Lab 6.0 software (Bio-Rad Laboratories, Hercules, CA, USA).

Table 1

Demographic, clinical and serologic characterization of shrimp allergic people.

Patient no.	Age	Shrimp-related symptoms	TM Special-IgE (kU _A /L)
1	15 years	asthma	1.98
2	13 years	bronchial asthma	2.35
3	9 years	leukocytosis	1.56
4	22 years	eczema	5.23
5	6 years	pneumonia	2.32
6	8 years	leukocytosis	2.36
7	14 years	rhinoconjunctivitis	1.38
8	25 years	cough variant asthma	2.35

The specific IgE of allergic serum > 0.35 (KU/L) was defined as effective.

2.5. Indirect ELISA

The IgG/IgE binding capacity of treated samples was assayed by ELISA (Li, Lu, Khan, Lin, & Zhang, 2014). Firstly, all the samples were diluted to 10 µg/mL using carbonate buffer solution (pH 9.6), and were coated in a 96-wells microtiter plate for 100 µL/well. After coating overnight and washing with PBST for three times, 1% BSA in PBST were used to block the plates. After washing the plates, rabbit polyclonal antibodies (IgG, 1: 10,000) and pooled patient sera (IgE, 1:100) were diluted using blocking buffer, respectively (Kamath, Abdel Rahman, Komoda, & Lopata, 2013). The plates were then loaded and incubated at 37 °C for 1.5 h. In addition, the plates were incubated with HRP-labelled goat anti-rabbit (IgG, 1:5000) or anti-human (IgE, 1:500) after washing again. Finally, TMB was added to each 100 µL/well and incubated at 37 °C for 20 min. The reaction was terminated with 2 M sulfuric acid. The absorbance of each well was measured at 450 nm. The experiment was repeated three times.

2.6. Structural analysis

2.6.1. Free amine analysis

Free amine content was detected as described by Blanco-Pascual, Fernández-Martín, and Montero (2013), with some modifications. L-leucine was used as the standard.

2.6.2. Available lysine analysis

Available lysine was detected following the protocols of Ou, Kwok, and Kang (2004), using DL-lysine as the standard.

2.6.3. Surface hydrophobicity

The surface hydrophobicity of EGCG-TM and CA-TM was analyzed following the protocols of Lin et al. (2015). The concentration of the samples was adjusted to 0.01–0.5 mg/mL in sequence, and ANS was used as a fluorescence probe. Surface hydrophobicity was defined as the fluorescence intensity of the resulting relative solutions under spectrophotofluorometry (Shimadzu, Kyoto, Japan).

2.6.4. Intrinsic fluorescence

The intrinsic fluorescence of EGCG-TM and CA-TM was detected with a F-4600 fluorescence spectrometer (Shimadzu, Kyoto, Japan) following Yuan et al. (2017). The parameters were set as follows: excitation 280 nm, emission 290–400 nm, scanning speed 1200 nm/s, and slit width 2.5 nm.

2.6.5. CD spectroscopy for secondary structures

Secondary structural changes of EGCG-TM and CA-TM were estimated as described by Wu, Wu, and Hua (2010). A Jasco J-815CD spectropolarimeter (Spectroscopic Co. Inc., Japan) was used for detection. The operating parameters were set as: 190–260 nm spectral range; 1.0 nm bandwidth; 100 nm/min scan rate; and 0.25 s interval. The samples were diluted to 0.5 mg/mL, and the percentage of the secondary structures was analyzed with the same software.

2.7. RBL-2H3 cell assay

RBL-2H3 cells were obtained from the Chinese Academy of Sciences, and were cultured following existing protocols (Lv et al., 2019). RBL-2H3 cells were cultured (5%CO₂ / 95% air atmosphere) in DMEM supplemented with 10% FBS, 100 U/mL penicillin and 100 µg/mL streptomycin at 37 °C. The cells were harvested when 2 × 10⁵ cells/mL density was reached, and were seeded in a 96-wells microtiter plate to measure the release of mediators and cytokines. RBL-2H3 cells were passively sensitized with mouse anti-TM IgE serum (1:10 dilution) for 15 h. They were then washed three times with Tyrode's buffer. β -hexosaminidase release was measured following Kuehn et al. (2010). Cells were stimulated with 500 ng/mL EGCG-TM and CA-TM in Tyrode's

buffer at 37 °C. Cells treated with Tyrode's buffer only were used as a negative control. All the samples were then centrifuged for 15 min. The release of histamine, cysteinyl leukotriene, prostaglandin D2, IL-4, and IL-13 was measured using ELISA kits.

2.8. In vivo analysis

2.8.1. BALB/c mice

Female BALB/c mice (5 weeks old, specific pathogen-free) were approved for use by Shanghai Laboratory Animal Center. The animals were raised in an air-conditioned room and allowed free access to tap water and food exclusive shrimp protein feed. All animal experiments were performed based on established guidelines by the Committee of Health Science Center of Shenzhen University, China (Permit No. 201911012).

2.8.2. Protocol for the allergy of mice model

The allergic mice model was carried out following a previously described method (Lv et al., 2019), with some modifications. For sensitization, intraperitoneal injection of mice was carried with 200 µg TM, EGCG-TM and CA-TM (n = 10) adsorbed in alum adjuvant (2 mg/ml) on days 7, 14, 21 and 28, as shown in Fig. 4A. After the sensitization stage, mice were separated into four groups (n = 10 per group; PBS, TM, EGCG-TM, and CA-TM) to document changes in allergenicity following different TM treatment. On days 31, blood samples were collected from all mice after sacrifice. Sera were obtained from the blood after centrifugation, and was stored at 20 °C until analysis. The level of TM-specific IgE and IgG1 serum was determined using indirect ELISA. The release of histamine and mMCP-1 in sera was measured using sandwich ELISA kits.

2.8.3. Spleen cell culturing and quantification of cytokine

Spleen cells were separated from mice to record changes to Treg, Th1, and Th2 cells following previously described method (Fei et al., 2016). Splenic lymphocytes were prepared aseptically using means of a cell strainer. The spleens were gently smashed, and the contents were centrifuged at 500 g for about 20 min. Afterwards, splenocytes were washed twice with RPMI-1640 medium. Then, the splenocytes were re-suspended and cultured in RPMI-1640 with 10% FBS and 1% PenStrep (penicillin-streptomycin solution) at a density of 2×10^5 cells/mL. The cells were re-stimulated with shrimp TM and subjected to different TM treatment for 72 h. Then, the cell supernatants were collected to

measure of cytokines IL-4, IL-13, and interferon (IFN)-γ by using ELISA kits.

2.9. Statistical analysis

All data were analyzed by Prism 8.0.2 software. The results are presented as mean ± SD. Differences were determined by ANOVA with SPSS Statistics (Chicago, USA). P-values ≤ 0.05 were considered statistically significant. Each experiment was repeated three times.

3. Results

3.1. Physicochemical characterization of conjugates

SDS-PAGE showed that the molecular weight changed after EGCG-TM and CA-TM (Fig. 1A). The band of native TM was ~36 kDa (Fig. 1A, Lane 1). The bands of EGCG-TM and CA-TM showed a slight upward migration (Fig. 1A, Lane 2 and 3), which meant that the molecular weight increased with the conjugation of TM and dietary polyphenols. The partial aggregation of bands was detected at ~72 kDa (Fig. 1A, Lane 2 and 3). The polyphenol content of EGCG-TM and CA-TM was increased (Table 2). The EGCG contents of the EGCG-TM complex

Table 2

The content of free amino and available lysine groups and secondary content by circular dichroism in shrimp tropomyosin (TM) and TM-polyphenol conjugates.

Protein/sample	TM	EGCG-TM	CA-TM
Polyphenones bound (mg/g)	–	31.68 ± 1.26	14.73 ± 0.87 ^c
Free amino group (µmol/mg protein)	4.16 ± 0.1	3.48 ± 0.08 ^a	3.72 ± 0.09 ^{b,c}
Available lysine group (g/100 g)	4.58 ± 0.11	3.67 ± 0.09 ^a	3.86 ± 0.12 ^{b,c}
Secondary content			
α-helix (%)	63.5 ± 0.1	42.6 ± 0.1 ^a	59.8 ± 0.2 ^{b,c}
β-sheet (%)	6.8 ± 0.2	11.7 ± 0.2 ^a	8.4 ± 0.1 ^{b,c}
β-turn (%)	13.8 ± 0.1	18.6 ± 0.3 ^a	14.6 ± 0.4 ^{b,c}
Random coil (%)	15.9 ± 0.3	27.1 ± 0.1 ^a	17.2 ± 0.1 ^{b,c}

Data are mean ± SD.

^a Statistical difference (p < 0.05) between TM-EGCG and control TM.

^b Statistical difference (p < 0.05) between TM-CA and control TM.

^c Statistical difference (p < 0.05) between TM-CA and TM-EGCG.

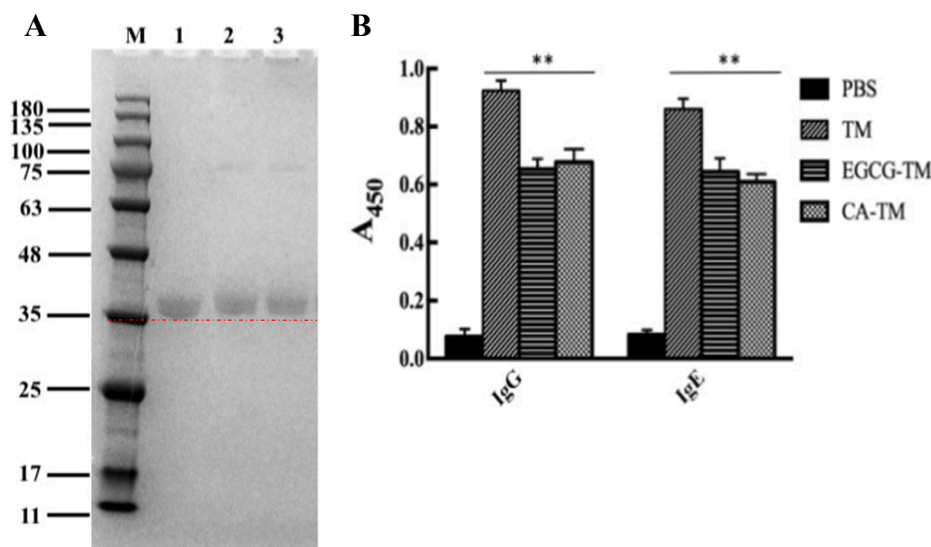


Fig. 1. Conjugation and IgG/IgE binding capacity of EGCG-TM and CA-TM. (A) Conjugation of EGCG-TM and CA-TM by SDS-PAGE analysis. An equal amount of TM (5 µg) was loaded into each lane. The molecular mass of the standard protein marker ranged from 11 to 180 kDa. M, protein marker; Lane 1, non-treated TM; Lane 2, EGCG-TM; Lane 3, CA-TM. (B) IgG/IgE binding capacity of EGCG-TM and CA-TM, and rabbit anti-TM IgG, or patient sera (IgE) used as the antibody.

and the CA contents of CA-TM complexes were 31.68 ± 1.26 mg EGCG/g and 24.73 ± 0.87 mg CA/g, respectively. These results confirmed the conjugation of TM with EGCG and CA, respectively. Free amine and available lysine of EGCG-TM and CA-TM decreased (Table 2). Free amine content declined by 16.3% and 9.6% after conjugation with polyphenols (EGCG and CA). Available lysine content declined by 19.9% and 15.7%, respectively. Both the free amine and available lysine of TM both decreased after conjugation with polyphenols, indicating that oxidized phenolic compounds can undergo a nucleophilic addition to the amino acid side chains, thus covalently modifying the proteins.

3.2. IgG/ IgE binding capacity

To determine whether TM-polyphenol complexes cause changes in immunoreactivity, the IgG/IgE binding capacity of EGCG-TM and CA-TM was evaluated by ELISA (Fig. 1B). The IgG-binding capacity of EGCG-TM and CA-TM decreased by $27.3 \pm 0.8\%$ and $24.8 \pm 0.5\%$, while the IgE-binding capacity decreased by $22.1 \pm 1.2\%$ and $25.1 \pm 0.6\%$, respectively. Thus, IgG/IgE binding to TM was decreased after conjugation with EGCG and CA.

3.3. Effect of structural changes of EGCG-TM and CA-TM

Surface hydrophobicity was higher for EGCG-TM and CA-TM compared to native TM (Fig. 2A). Compared with the native TM, EGCG-TM showed a ~3-fold increase in surface hydrophobicity. CA-TM also showed a similar result. However, surface hydrophobicity was higher for EGCG-TM compared to CA-TM. The maximum emission spectrum of TM was ~308 nm (Fig. 2B). The maximum fluorescence intensity was lower for EGCG-TM and CA-TM compared to native TM. The maximum emission wavelength of EGCG-TM and CA-TM shifted towards the red spectrum from 308 to 313 nm, after modification. Compared with native TM, EGCG-TM had the lowest intrinsic fluorescence intensity (about 63.2%).

The CD spectra for different treated TM are shown in Fig. 2C. TM spectra exhibited one positive peak (~196 nm) and two negative peaks (~212 and ~223 nm). The intensity steadily decreased with EGCG and CA treatment. JASCO secondary structure software showed that native TM had 63.5% α -helix, 6.8% β -sheet, 13.8% β -turn, and 15.9% random coil content (Table 2). After conjugation with EGCG and CA, α -helix content significantly decreased.

3.4. The effect of EGCG-TM and CA-TM on the release of mediators and cytokines from IgE-sensitized RBL-2H3 cells

The release of β -hexosaminidase decreased more (60.4% and 36.7%) in cells exposed to EGCG-TM and CA-TM compared with TM alone (Fig. 3A). In addition, EGCG-TM and CA-TM caused histamine release to decline (56.0% and 34.8%) (Fig. 3B). The release of cysteinyl leukotriene and prostaglandin D₂ also followed a similar trend (Fig. 3C and 3D).

RBL-2H3 cells can produce cytokines such as IL-4 and IL-13. They might have a pathological effect on IgE-mediated allergic symptoms, including atopic dermatitis, atopic rhinitis, and asthma. EGCG-TM and CA-TM caused a significant decrease in the release of IL-4 release (48.6% and 29.2%) and IL-13 release (49.6% and 26.9%) (Fig. 3E and F).

3.5. Sensitization potentials following intraperitoneal exposure to EGCG-TM and CA-TM

The BALB/c mice model was used to test the allergenicity of EGCG-TM and CA-TM after i.p. injection (Fig. 4A). Exposure to EGCG-TM and CA-TM caused TM-specific IgE and IgG1 levels to decline. This decline was 2- to 3-fold more for EGCG-TM and CA-TM compared to TM alone (Fig. 4B and 4C). Serum histamine levels was significantly decreased with EGCG-TM and CA-TM (Fig. 4D), with a similar trend occurring for

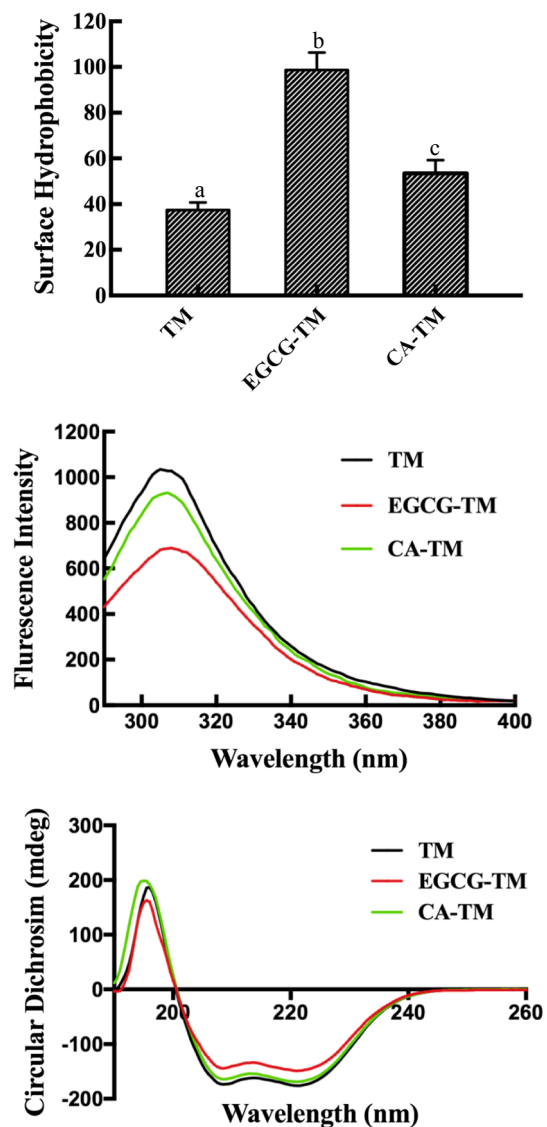


Fig. 2. (A) Surface hydrophobicity (B) intrinsic fluorescence and (C) CD spectroscopy findings with EGCG-TM and CA-TM treatment.

mMCP-1 levels (Fig. 4E). The production of IL-4 and IL-13 also decreased with EGCG-TM and CA-TM (Fig. 4F and 4G). However, IFN- γ levels were higher with EGCG-TM and CA-TM compared to TM alone (Fig. 4H).

4. Discussion

When polyphenols interact with food allergens, they cause structural changes to proteins, which might influence the potential allergenicity (Lu et al., 2018; Zhang et al., 2020). The current study used the free radical method to prepare TM-polyphenol covalent complexes. Hydroxide free radicals were generated by oxidation-deduction reactions between H₂O₂ and ascorbic acid, which might attack the amino complexes in proteins to generate radical species. Phenolic compounds then react with these radical species (Liu et al., 2017). SDS-PAGE results showed that the molecular weight of TM-polyphenol conjugation significantly increased, with slight cross-linked to generate protein-polyphenol aggregation. The polyphenol content of EGCG-TM and CA-TM was increased, while free amine and available lysine contents declined after conjugation with polyphenol (EGCG and CA). Thus, covalent interactions might occur between polyphenols (EGCG and CA) and TM. Our findings support existing studies, which demonstrated

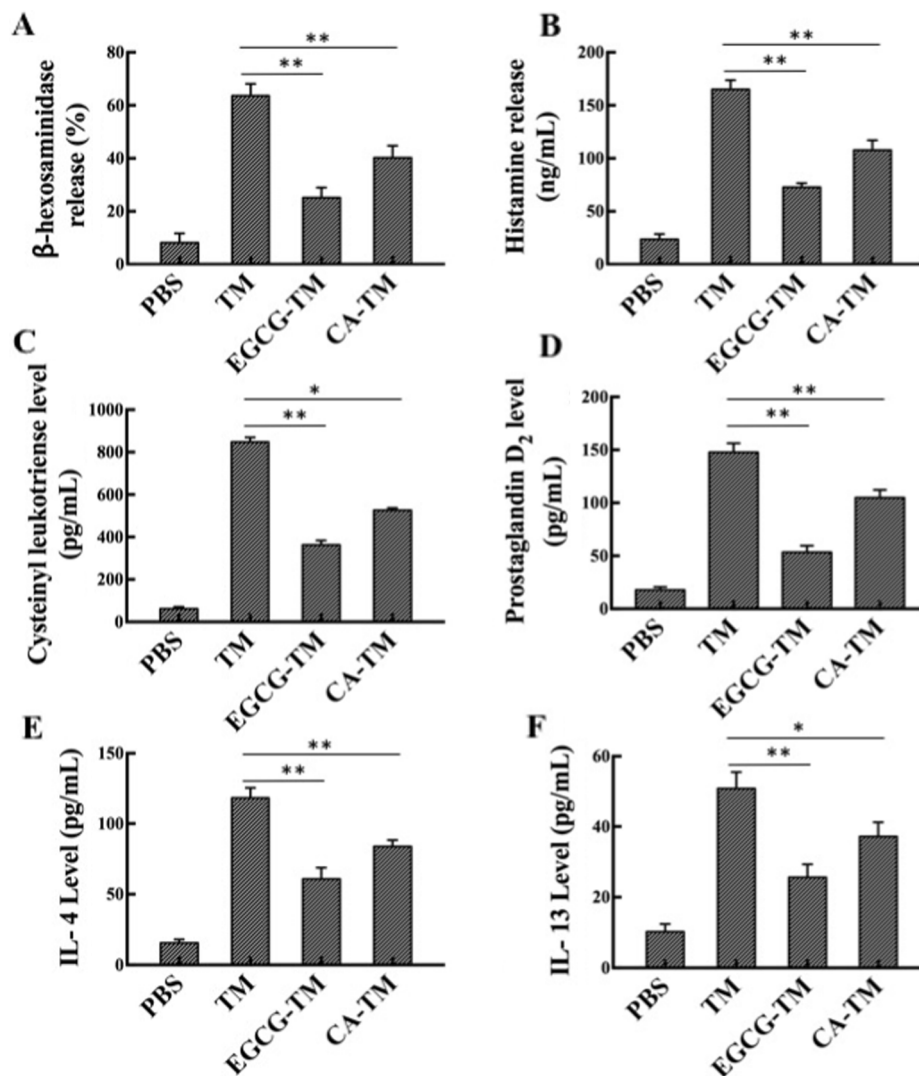


Fig. 3. Effect of EGCG-TM and CA-TM on IgE-mediated allergic responses in RBL-2H3 cells sensitized with mouse serum IgE. Release of A, β -hexosaminidase; B, histamine; C, cysteinyl leukotriene; D, prostaglandin D₂; E, IL-4; and F, IL-13. * $p < 0.05$, ** $p < 0.01$. The data represent the mean $\pm$ SD from three independent experiments.

covalent interactions between chlorogenic acid and ovalbumin (Lu et al., 2018), covalent modifications of whey protein isolate by EGCG (Jia et al., 2016), the covalent interaction between milk whey protein and coffee-specific phenolics (Ali, Homann, Khalil, Kruse, & Rawel, 2013), and covalent interactions between proteins and oxidation products of caffeoylquinic acid (Prigent, Voragen, Visser, van Koningsveld, & Gruppen, 2007). TM-polyphenol complexes might also reduce the IgG/IgE binding capacity of TM on indirect ELISA. The modification of amino acid side chains on the TM structure might contribute to the destruction of TM allergic epitopes and loss of IgG/IgE binding capacity, potentially altering the molecular properties of TM.

The extent to which the potential allergenicity of TM declined might be relative to structural changes. Therefore, the current study used surface hydrophobicity and intrinsic fluorescence to evaluate the tertiary structure of EGCG-TM and CA-TM samples. Increased in surface hydrophobicity might be caused by the exposure of hydrophobic groups or hydrophobic interactions with the treatment. Treated TM caused more hydrophobic groups to be exposed to the reaction of hydroxyl groups and certain amino acid residues, thus increasing the surface hydrophobicity. Previous studies reported that the hydrophobicity of soy protein increases with anthocyanin treatment (Sui et al., 2018). Tertiary structural changes to TM are influenced by the interaction of

polyphenols with amino acid residues (Lv et al., 2018). The phenomenon indicates that the binding capacity of IgG/IgE with TM-polyphenol complexes decreases.

Intrinsic fluorescence of protein was determined by typical fluorescence amino acids. The fluorophores of protein contain tryptophan and tyrosine residues as a fluorescence emission spectrum and are responsible for causing variation in the spectra (Chen et al., 2018). However, Trp does not exist in shrimp TM (Zheng, Lin & Pawar, 2011). Therefore, Tyr residues could be used to analyze changes to intrinsic fluorescence. The maximum emission wavelength for TM was ~ 308 nm, which might be attributed to Tyr residues. Moreover, fluorescence intensity was lower for TM-polyphenol complexes compared to TM, indicating that TM-polyphenol complexes quench fluorescence. A slight red shift of the spectrum was also observed, which might be attributed to changes in the aromatic amino acid (tyrosine residues) microenvironment. Prigent et al. found that the side chains were more reactive to tyrosine and lysine than histidine and tryptophan in covalent interactions between caffeoylquinic acid and proteins (Prigent et al., 2007). These changes might be caused by reduced exposure of amino acids to the aqueous environment, because specific amino acid residues are modified after TM-polyphenol complexes are formed (King & Li, 1999). Reduced fluorescence intensity with EGCG-TM and CA-TM might reveal change to the

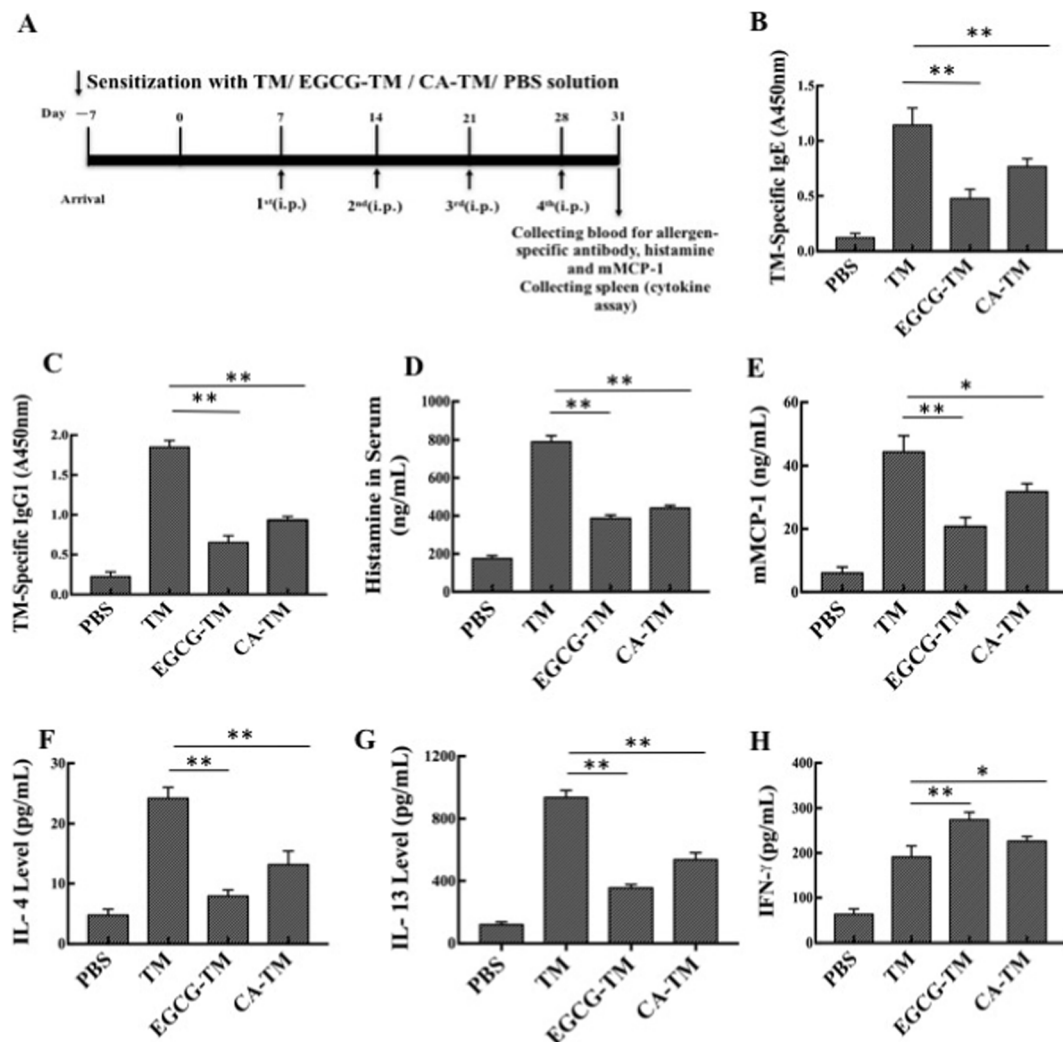


Fig. 4. Sensitization experiment. A, sensitization protocol; B-C, levels of IgE and IgG1 measured on day 31; D, release of histamine measured in serum from mice; E, release of mouse mast-cell protease-1 (mMCP-1) measured in serum from mice; F-H, production of IL-4, IL-13 and IFN- γ measured in spleen cell culture. * $p < 0.05$, ** $p < 0.01$. The data represent the mean $\pm$ SD from triplicate determinations.

alteration in the structure of the allergic proteins.

The structure of EGCG-TM and CA-TM was further analyzed by CD spectroscopy. CD spectroscopy revealed the secondary structure of the protein, which contained α -helix, β -sheet, and β -turn content, and a random structure (Li, Lin, Limin, & Jamil, 2007). α -helix content was represented by two negative peaks. β -sheet content was represented by a positive peak. The positive and negative peaks both decreased after TM-polyphenol complexes were formed. When α -helix and β -sheet content decreased, the secondary structure became considerably disordered, and was rearranged. This phenomenon might be explained by TM-polyphenol complexes causing the protein structure to unfold more. For instance, covalent conjugation with EGCG and chlorogenic acid creates an unfolded OVA structure (Lu et al., 2018; He et al., 2019). TM-polyphenol complexes might destroy the allergic epitope, due to the changes in the linear and conformational structures. Therefore, the tertiary structure might be disrupted due to the secondary structure changing as a consequence of the forming of TM-polyphenol complexes (Shin, Han, & Ahn, 2013).

IgE-mediated RBL-2H3 cells could be used to assay type I allergic hypersensitivity reactions (Zhou, Zhao, Cui, & Sun, 2015). Cellular degranulation components (including active mediators and cytokines) are important components that contribute to the expression of immunoreactivity and pathogenesis caused by allergenic protein. These assays are commonly used to examine the variations in the IgE bindings and

their ability to induce clinical sensitization. EGCG-TM and CA-TM could significantly decrease the release of mediator and cytokines, potentially suppressing the onset of allergic reactions. The ability of the allergenic proteins to secrete mediators and cytokines is also reduced by different processing methods (Ahmed et al., 2019; Zhang et al., 2019). TM-polyphenol complexes might alter the IgE epitopes in RBL-2H3 cells, thus hindering successful basophil degranulation. Therefore, TM-polyphenol complexes could mitigate potential allergenicity.

The current study used BALB/c mice to evaluate the potential allergenicity of EGCG-TM and CA-TM. Allergic sensitization was lower in EGCG-TM and CA-TM compared to TM, as demonstrated by a lower level of TM-specific IgG1 and IgE in the immunogenic response. Furthermore, EGCG-TM and CA-TM reduced the capacity to induce the release of mMCP-1 and histamine in serum. Similar results were obtained when evaluating the allergenic potential of mice using different processing methods (Long, Yang, Wang, Hu, & Chen, 2015; Xu et al., 2017). Moreover, changes to cytokine production were also determined for the immune responses using the mouse model. TM-polyphenol complexes had significantly lower IL-13 and IL-4 levels compared to the native TM. In comparison, IFN- γ levels increased. IFN- γ is produced by Th1 cells, while IL-13 and IL-4 are secreted by Th2-type cells (Shi et al., 2015). The detection of these cytokines indicated that TM-polyphenol complexes might promote the Th1 immune response and suppress the Th2 immune response, which, in turn, suppresses the TM

allergic reaction. Moreover, IL-13 might influence IgE production and IgE-mediated allergic responses (Jan, de, & Vries, 1998). Reduced IL-13 levels in the current study indicated that conjugation with EGCG and CA impact the allergenicity of TM. This result supported the detection of IgE in mice. The response of TM-polyphenol complexes in mitigating allergenicity might be attributed to the changes in their ability to binding epitopes on the surface of EGCG-TM and CA-TM.

5. Conclusion

The current study elucidated changes to the allergenicity changes of TM after conjugation with EGCG and CA *in vitro* and *vivo*. The formation of shrimp TM-polyphenol complexes caused surface hydrophobicity to increase and fluorescence intensity to decrease. Consequently, the secondary structure became considerably disordered. TM-polyphenol complexes induced structural changes to TM, destroying the allergic epitopes. As a result, IgG/IgE binding capacity decreased with the formation of TM-polyphenol complexes. In addition, EGCG-TM and CA-TM caused the release of mediators from stimulated cells to decline. EGCG-TM and CA-TM could change the conformational structure of the protein, mitigating potential allergenicity and diminishing the risk of shrimp TM to human health. The study provided a novel method for reducing the allergenicity of TM, which might have potential in producing hypoallergenic food, although clinical trials need to be performed to validate the allergenic properties.

CRedit authorship contribution statement

Liangtao Lv: Investigation, Validation, Writing - original draft, Writing - review & editing. **Xin Qu:** Investigation, Methodology, Validation. **Ni Yang:** Resources, Supervision. **Zhigang Liu:** Supervision, Writing - review & editing, Funding acquisition, Project administration. **Xuli Wu:** Conceptualization, Funding acquisition, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodres.2020.109997>.

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Pooling of sera from patients with allergy to shrimp

Serum samples from 8 shrimp-allergic patients were tested in this study. Serum-specific IgE antibodies to shrimp tropomyosin (TM) were analyzed by ImmunoCAP system (Phadia AB, Uppsala, Sweden). All selected serum samples with Special-IgE values higher than or equal to 0.5 kU_A/L were pooled. [1]
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Table 1. Demographic, clinical and serologic characterization of shrimp allergic people

Patient no.	Age	Shrimp-related symptoms	TM Special-IgE (kU _A /L)
1	15 years	asthma	1.98
2	13 years	bronchial asthma	2.35
3	9 years	leukocytosis	1.56
4	22 years	eczema	5.23
5	6 years	pneumonia	2.32
6	8 years	leukocytosis	2.36
7	14 years	rhinoconjunctivitis	1.38
8	25 years	cough variant asthma	2.35