



Research Article

Allergenicity suppression of tropomyosin from *Exopalaemon modestus* by glycation with saccharides of different molecular sizes

Ziye Zhang^{a,b}, Hang Xiao^b, Peng Zhou^{a,*}

^a State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu Province 214122, China

^b Department of Food Science, University of Massachusetts, Amherst, MA 01003, USA



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ABSTRACT

The allergenicity suppression of tropomyosin (TM) from *Exopalaemon modestus* by glycation with saccharides of different molecular sizes (glucose, maltose, maltotriose, maltopentaose and maltoheptaose) was investigated using immunoblotting, human colon epithelial cell line (Caco-2) and human basophil cell line (KU812). Glycation of TM by glucose, maltotriose, maltopentaose and maltoheptaose significantly destructed and masked TM epitopes to obtain lower allergenicity, while glycation of TM by maltose had insignificant suppression on TM allergenicity. In addition, the glycated TM by glucose, maltotriose, maltopentaose and maltoheptaose inhibited the proliferation and IL-8 secretion of Caco-2, and the CD63 and CD203c expression, MAPK signaling of KU812 basophils, while the glycated TM by maltose had insignificant suppression on the allergy reactivities of Caco-2 cells and KU812 basophils. Glycation of TM by saccharides with larger molecular sizes (such as maltoheptaose) could provide new insight into the desensitization of shrimp-induced food allergy.

1. Introduction

Exopalaemon modestus (EM, the freshwater Siberian prawn) is a famous shrimp delicacy in China, while could trigger severe food allergy. In recent years, EM has been introduced to the Western Coast of North America (California, Oregon and Washington, Emmett, Hinton, Logan, & McCabe, 2002; Young, Berridge, O'Rear, Moyle, & Durand, 2017), which makes it become a potential new allergen. The allergen of EM is Exo m 1, a tropomyosin (TM). Due to the increased consumption of EM in China, the occurrence of EM-induced food allergy reactions also got promoted in recent years.

Glycation could modify the allergenicity of allergens, and different saccharides could exert different desensitization effects on allergens during glycation (Kobayashi et al., 2001; Nakamura, Watanabe, Ojima, Ahn, & Saeki, 2005). TM is lysine rich, glycation could modify TM conformation and therefore reduce allergenicity. However, how glycation of TM by saccharides with different molecular sizes affects the allergenicity of TM still remains unwell-known. Besides, food allergens are resistant to gastrointestinal digestion, therefore remains allergen fragments with epitopes in digestive tracts to trigger allergic reactions (Astwood, Leach, & Fuchs, 1996). However, there were few data on how glycation affected the allergenicity of TM gastrointestinal digests.

Basophils are pivotal cells in triggering food allergy due to the fact that they could secrete immune mediators (such as cytokines and

histamine). The activation of basophils involves CD antigens expression (such as CD63 and CD203c) and MAPK (mitogen-activated protein kinases) signaling pathways (Liu et al., 2016). CD antigens and MAPKs signaling cascades are important in allergy activation and degranulation of basophils (Christensen et al., 2014; Kneidinger et al., 2008; Liu et al., 2016; Qi & Elison, 2005), while how glycated TM affected basophil activation is still unwell-known.

Reducing TM allergenicity is beneficial to shrimp allergy patients. The objectives of this study are to investigate the allergenicity of TM and glycated TM, as well as their allergy sensitization on human cells [colon epithelial cell line (Caco-2) and basophil cell line (KU812)]. These results would improve the understanding of how saccharides with different molecular sizes affected TM allergenicity by glycation with saccharides of different molecular sizes, which would provide a new insight into the preventive and desensitization of shrimp induced food allergy.

2. Materials and methods

2.1. Materials

The purification of shrimp TM from *Exopalaemon modestus* (Exo m 1) was according to Zhang, Zhang, Chen, and Zhou (2018). The sera of shrimp allergy subjects were from Wuxi People's Hospital (Table 1,

* Corresponding author.

E-mail address: zhoupeng@jiangnan.edu.cn (P. Zhou).

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Table 1
Information of shrimp allergy patients' sera.

Subjects	Gender	Age (year)	Clinical symptoms	IgE (kU _A /L)
1	Male	3	AS, HI	29.5
2	Female	2	AS	23.2
3	Female	47	HI, EC	11.6
4	Male	4	AS, HI	65.5
5	Female	40	HI, CW	11.4
6	Female	14	HI, NW	22.7
7	Male	5	EC, RH	35.1
8	Male	2	AS, NW	35.9

Note: AS, asthma; CW, chest wheezing; EC, eczema; HI, hives; NW, nose wheezing; RH, rhinitis.

Wuxi, Jiangsu, China). 2',7'-dichlorodihydrofluorescein diacetate (H₂DCF-DA, CAS: 4091-99-0), D-glucose (G, CAS: 50-99-7), maltose (M, CAS: 6363-53-7), maltotriose (MTS, CAS: 1109-28-0), maltoheptaose (MHS, CAS: 34620-78-5), pepsin (CAS: 9001-75-6) and trypsin (CAS: 9002-07-7) were purchased from Sigma-Aldrich Inc. (MO, USA). Maltoheptaose (MPS, CAS: 34620-76-3) was obtained from Miragen Inc. (DE, USA). The human colon epithelial cell line (Caco-2) and human basophil cell line (KU812) were obtained from Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China).

2.2. TM glycation

TM was dissolved in a solution (1 mM NaHCO₃ and 5 mM β-Mercaptoethanol) containing glucose, maltose, maltotriose, maltopentaose, maltoheptaose (10.8%, w/v), respectively, with TM concentration of 0.2 mg/mL. Each milliliter of the mixture was lyophilized. The lyophilized mixtures were incubated in a desiccator containing saturated aqueous Mg(NO₃)₂ solution at 60 °C for 48 h, to form the glycated TM by glucose maltose, maltotriose, maltopentaose and maltoheptaose (TM-G, TM-M, TM-MTS, TM-MPS, TM-MHS), then cooled to room temperature (25 °C). Afterwards, glycated TMs were transferred to dialysis tubing (10 kDa, Union Carbide Corp., Danbury, CT, USA), dialyzed in ultrapure water at 4 °C for 48 h to remove extra saccharides, lyophilized prior to analysis. The glycation degree of glycated TMs was determined by molecular weight changes using SDS-PAGE.

2.3. Circular dichroism

The circular dichroism (CD) spectra were recorded using a CD spectrometer (MOS-450, Bio-Logic Science Instruments, France). The secondary structures were analyzed using DichroWeb (<http://dichroweb.cryst.bbk.ac.uk>).

2.4. Allergenicity analysis of TM and glycated TM

Western blot: Firstly, the TM and glycated TMs were electrophoretic separated using SDS-PAGE, then proteins were transferred to a PVDF membrane and incubated in a blocking solution (5% defatted milk). Afterwards the membrane was moved in a diluted sera (1:10 diluted with 1% defatted milk), incubated at 4 °C. The membrane was then incubated with horseradish peroxidase (HRP)-conjugated goat anti-human IgE (KPL Inc., MD, USA). The protein-antibody complex in membrane was detected with ECL luminous and determined using a ChemiDoc XRS⁺ imager (Bio-Rad, CA, USA).

ELISA: The TM and glycated TM samples (100 μL/well) were coated on 96 well plates and incubated at 37 °C for 2 h. Then blocked with blocking solution (200 μL/well), incubated with diluted human sera and HRP-goat anti-human IgE at 37 °C. The color was developed using TMB solution and terminated using 2 M H₂SO₄ (50 μL/well), the absorbance (OD_{450 nm}) was measured using a plate reader (SpectraMax M5, Molecular Devices, Sunnyvale, CA, USA).

2.5. In vitro gastrointestinal digestion

For gastric digestion, TM and glycated TM was incubated with pepsin (pepsin: TM = 1:50, w/w) at 37 °C. At 0, 5, 15, 30, 40, 50 and 60 min, 100 μL gastric digest was transferred to a microtube and stopped digestion with 30 μL 0.2 M Na₂CO₃. As to the simulated intestinal digestion, the TM and glycated TM were incubated with trypsin (trypsin: TM = 1:100, w/w) at 37 °C. At different time, 100 μL digest was transferred to a tube and digests were heated in boiling water to terminate digestion. The gastrointestinal digestion was performed as following: after gastric digestion, the gastric digest pH was brought to 7.5, then going on the simulated intestinal digestion. The allergenicity of digests was analyzed using ELISA.

2.6. The human colon epithelial cell model assay

2.6.1. Proliferation determination

Caco-2 cells (1 × 10⁵ cells/well) were seeded on 96 well plates and cultured in DMEM medium. Then cells were washed three times with PBS and incubated with TM or glycated TM at 37 °C for 24 h. Afterwards 10 μL CCK-8 were plunged into each well and incubated for 60 min, the absorbance (OD_{450 nm}) was measured by a plate reader.

2.6.2. IL-8 secretion determination

Caco-2 cells were washed with pre-warmed PBS (37 °C). To measure the release of IL-8 cytokine, Caco-2 cells were incubated with TM or glycated TM (37 °C for 24 h). Afterwards, the IL-8 release was measured using a commercial human IL-8 ELISA kit (R&D systems, Minneapolis, MN, USA).

2.7. The human basophil cell model assay

2.7.1. Preparation of IgE-sensitized basophil

KU812 cells (1 × 10⁵ cells/well) were cultured on a 96 well plate in IMDM medium cultured (37 °C for 24 h). Afterwards, cells were washed three times with PBS and sensitized using patients' sera diluted in medium without FBS (1:10, v/v). After incubation for 24 h, basophils were washed with PBS for three times. Basophil allergy activation was activated by incubating with TM or glycated TM (100 μL/well, 60 min).

2.7.2. Histamine release assay

The supernatant of culture medium from IgE-sensitized KU812 basophils (50 μL/well) was used to determine the histamine release using a commercial histamine ELISA kit (IBL International, Germany).

2.7.3. Degranulation determination

The basophil degranulation was performed with determining the β-hexosaminidase in culture medium: 10 μL supernatant of IgE-sensitized basophils was plunged into a 96-well plate to each well, and then 50 μL 4-nitrophenyl N-acetyl-β-D-glucosaminide (1 mM in 0.1 M citric acid buffer, pH 4.5) was moved to each well. After incubation (37 °C for 60 min), 0.2 M glycine solution (pH 10.7, 100 μL/well) was added and absorbance was determined (OD_{450 nm}). The degranulation was performed as the β-hexosaminidase activity measured in supernatant samples compared with the total levels in the corresponding cell lysates. For determining the total activity, the cells were lysed with Triton-X 100 (0.1%) before removing supernatant. The β-hexosaminidase activity was quantified by measuring the hydrolyzed substrate using a plate reader.

2.7.4. Reactive oxygen species (ROS) determination

The IgE-sensitized KU812 basophils (1 × 10⁵ cells/well) were incubated in 5 μM of H₂DCF-DA (37 °C for 30 min), then cells were washed to remove the extra H₂DCF-DA, and treated with TM or glycated TM for 30 min. The fluorescence intensity was determined at excitation (485 nm) and emission (530 nm) using a fluorometer (SpectraMax M5,

Molecular Devices, USA).

2.7.5. Measurement of IL-4 and TNF- α release

For cytokine measurement, the supernatant of IgE-sensitized basophils was used to determine IL-4 and TNF- α release, using the human IL-4 and TNF- α ELISA kit (R&D system, USA) using the supernatant of culture media harvested at 4 h and 6 h, respectively.

2.7.6. Flow cytometry assay

The IgE-sensitized KU812 basophils were centrifuged and washed three times with PBS. Adding 100 μ L flow cytometry staining buffer (eBioscience, Thermo Scientific, IL, USA) to the washed cells, and separated to two equal parts, adding FITC Mouse Anti-Human CD63 and PE Mouse Anti-Human CD203c (BD Bioscience, MA, USA), respectively, then incubated in dark for 30 min. Washing the cells three times and resuspended using 300 μ L staining buffer, the expression of surface antigens (CD63 and CD203c) was analyzed using a FACSCalibur flow cytometer (BD Biosciences, USA).

2.7.7. Proteome profiler array

The IgE-sensitized KU812 cells were washed with PBS. Adding lysis buffer 6 to lyse cells with gentle shake (4 °C for 30 min), then cells were centrifuged to get supernatant, and the protein concentration was determined using BCA protein assay kit (Thermo, USA) and adjusted to 1 mg/mL. The relative phosphorylation levels of MAPK signaling proteins were determined using human phospho-MAPK array kit (R&D systems, MN, USA). The MAPK proteins expression was compared with the IgE stimulated basophils (Incubated human IgE (Abcam) for 3 h and subsequently stimulated with anti-IgE (Abcam) for 30 min).

2.8. Statistical analysis

Experimental data were presented as means $\pm$ standard deviation. Statistical analysis was carried out using SPSS software (SPSS Inc., Version 19, IL, USA).

3. Results and discussion

3.1. Allergenicity of TM and glycated TM

Glycation could modify TM conformation therefore affect allergenicity (Nakamura et al., 2005; Yang, Li, Li, & Wang, 2013). The glycation degree of TM was shown in Fig. 1A, compared to TM (lane 1), the molecular weight increased as: TM-MTS (lane 4) > TM-G (lane 3) $\approx$ TM-M (lane 2) > TM-MPS (lane 5) > TM-MHS (lane 6) > TM (lane 1), which suggested TM could be highly glycosylated by saccharides with smaller molecular size (glucose, maltose and MTS), while less glycosylated by saccharides with larger molecular sizes (MTS, MPS and MHS), which might be due to that shorter saccharide chains had less steric hindrance and thus were more likely to react with TM, while longer saccharide chains (MPS and MHS) had more steric hindrance to react with TM, resulted in lower glycation degree. Previous data also showed similar results. Nakamura et al. (2005) reported that after glycation, the scallop tropomyosin glycosylated by glucose, maltose and maltotriose performed lower glycation extent and available lysine content with the increased molecular size of reducing sugars, Chen, Liu, Labuza, and Zhou (2013) found that increasing molecular size of reducing sugars (glucose, maltose and maltotriose) reduced the glycation extent on β -lactoglobulin, Zhang et al. (2014) reported the glycation degree of α -lactalbumin decreased with an increased size of saccharides (glucose, maltose, maltotriose, maltotetraose and maltopentaose).

As to pinpoint the effects of glycation on TM conformation, the secondary structures of TM were investigated. As shown in Fig. 1B, TM had α -helix (78.4%), β -turn (5.2%) and random coils (14.4%), which were similar with previous data (Zhang et al., 2018). After glycation, the α -helix decreased from 78.7% (TM) to 18.5% (TM-G), 17.8% (TM-

M), 15.6% (TM-MTS), 25.4% (TM-MPS) and 40.5% (TM-MHS), while β -sheets improved from 0% (TM) to 20.3% (TM-G), 25.7% (TM-M), 31.4% (TM-MTS), 21.5% (TM-MPS) and 16.3% (TM-MHS), β -turns rose from 5.1% (TM) to 21.4% (TM-G), 23.2% (TM-M), 30.5% (TM-MTS), 25.7% (TM-MPS) and 20.6% (TM-MHS), and random coils promoted from 14.2% (TM) to 39.8% (TM-G), 42.5% (TM-M), 43.7% (TM-MTS), 38.4% (TM-MPS) and 33.8% (TM-MHS), respectively. These data showed that glycation led to transfer of α -helix to other structures, led to the instability of TM conformation.

The allergenicity of TM and glycated TM was investigated using western blot (Fig. 1C) and ELISA (Fig. 1D) with patients' sera. Compared with TM (Fig. 1C, lane 1), the immunoblotting intensity of TM-G (lane 2), TM-MTS (lane 4), TM-MPS (lane 5) and TM-MHS (lane 6) got significantly weaker, while TM-M (lane 3) had insignificant decrease. The ELISA data (Fig. 1D) had similar results with western blot. Lysine, an amino acid with free ϵ -amino group residue, mostly participated in food allergen glycation. Besides lysine, arginine and histidine (which have free ϵ -amino group) were found to participate in glycation process (Arena, Salzano, Renzone, D'Ambrosio, & Scaloni, 2014; Gupta et al., 2018). As lysine, arginine and histidine were participants during protein glycation, and all the three amino acids existed in TM epitopes (Ayuso, Lehrer, & Reese, 2002), the TM epitopes with lysine, arginine or histidine could be destroyed and masked by saccharide residues on TM surface to reduce their affinity to IgE binding, resulted in lower allergenicity of TM-G, TM-MTS, TM-MPS and TM-MHS. While compared with TM, the TM-M had relatively lower reduction of allergenicity (Fig. 1C, D), this might be due to that, although glycation could destroy and mask epitopes, glycation could lead to the formation of TM adducts with various glycosylated structures such as N $^{\epsilon}$ -(carboxymethyl)-lysine (CML), known as advanced glycation end products (AGEs, Gupta et al., 2018). Among various AGEs, CML has been proved to function as neoallergens with additional IgE binding property to strengthen allergenicity (Ikeda et al., 1996; Mueller et al., 2013; Gupta et al., 2018). The generation of neoallergens might partly offset the TM epitope loss, therefore resulted in the insignificant allergenicity changes of TM-M.

3.2. The allergenicity of TM and glycated TM gastrointestinal digests

As TM was resistant to gastrointestinal digestion, in order to investigate how glycation of TM by saccharides with different molecular sizes affected the allergenicity of digests, the allergenicity of gastric, intestinal and gastrointestinal digests of TM and glycated TM were analyzed in this part.

As for gastric digestion (Fig. 2A), the allergenicity (OD_{450 nm}) of all digests declined, indicating the TM epitopes could be hydrolysis-destroyed by pepsin. While the gastric digests were still with a relatively IgE-reactivity after gastric digestion for 1 h, suggesting pepsin could not enzymatically destroy epitopes which might be due to the low hydrolysis site contents (Zhang, Xiao, Zhang, & Zhou, 2019). As to glycated TM, the absorbance of gastric digests varied as following: TM > TM-M > TM-G > TM-MTS > TM-MPS > TM-MHS, which was inversely consistent with the glycation degree (Fig. 1A), suggested the less glycosylated TM (TM-MHS) could be more likely to be hydrolyzed, this might be due to that: (1) less glycation had weaker masking effects of saccharide residues on TM epitopes, therefore epitopes were more likely to be hydrolyzed; (2) the relatively lower glycation could loosen TM conformation to make pepsin easier accessible to TM hydrolysis sites. Thereby, relatively lower glycation of TM by MHS could both mask/destroy TM epitopes and destabilize TM conformation to expose hydrolysis sites and make it more likely to be hydrolyzed by pepsin, reaching a synergetic effect of epitope masking and conformation instability to hydrolyze the glycosylated TM, leading to the gastric digests with relatively lower allergenicity.

As for intestinal digestion (Fig. 2B), the allergenicity of intestinal digests decreased sharply as digestion lasted longer time, suggesting that most epitopes were hydrolyzed because of the high TM tryptic-

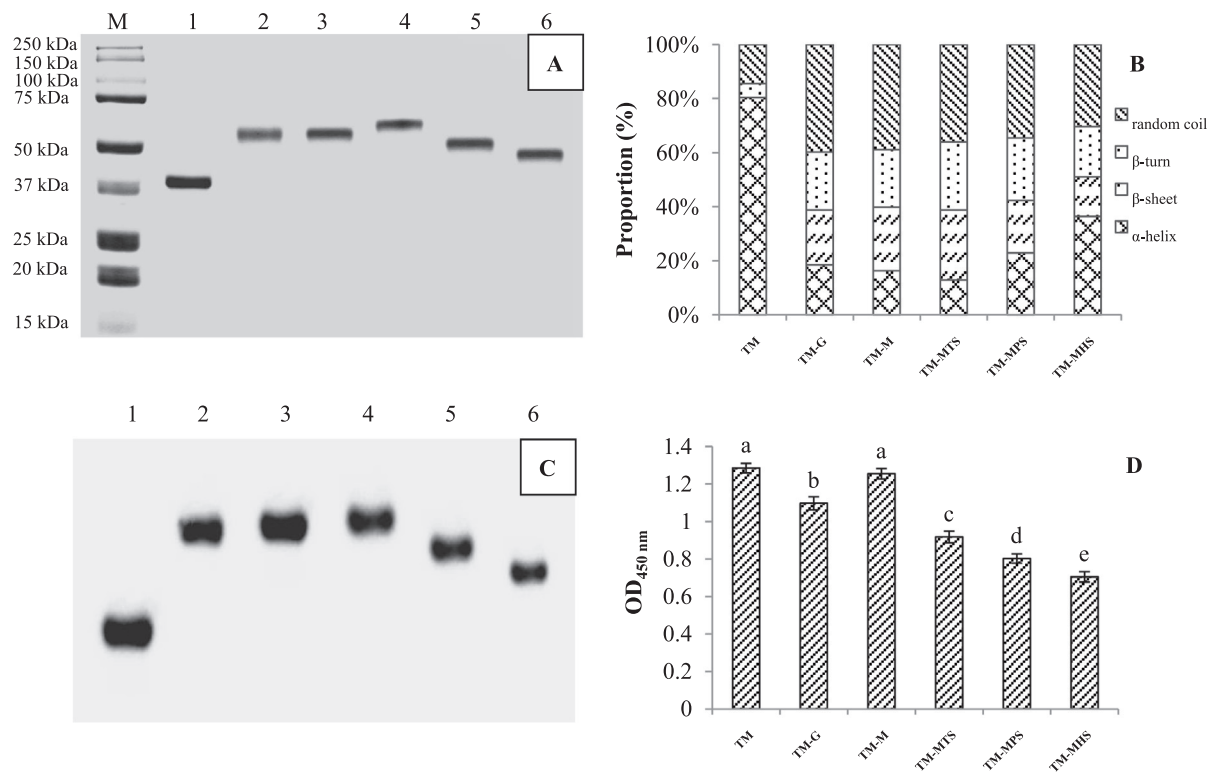


Fig. 1. The immunoblotting (A) and allergenicity (B), SDS-PAGE (C) and secondary structure analysis (D) of TM and glycosylated TM. Note: A: M, marker; lane 1, TM; lane 2, glucose glycosylated TM; lane 3, maltose glycosylated TM; lane 4, maltotriose glycosylated TM; lane 5, maltopentaose glycosylated TM; lane 6, maltoheptaose glycosylated TM. C: lane 1, TM; lane 2, glucose glycosylated TM; lane 3, maltose glycosylated TM; lane 4, maltotriose glycosylated TM; lane 5, maltopentaose glycosylated TM; lane 6, maltoheptaose glycosylated TM.

digestion sites (Zhang et al., 2019). Also, as to the glycosylated TM, the allergenicity of digests increased with higher TM glycosylation degree, while decreased with lower TM glycosylation degree. Which might be resulted from that higher glycosylation could induce greater conformational changes, with more tryptic hydrolysis sites exposed and hydrolyzed. However, the allergenicity of intestinal digests was relatively high even after digestion went to 180 min. Saccharide residues could block the interactions between TM and trypsin, leading to the digests with allergenicity.

As for gastrointestinal digestion, Fig. 2C suggested that after gastric digestion, there were allergenic peptides with epitopes in the gastric digests. The subsequent tryptic-digestion highly destroyed the remained epitopes, led to digests with lower allergenicity. Although TM could be hardly hydrolyzed by gastric digestion, the subsequent tryptic-hydrolysis actions were efficacious to hydrolyze the remained epitopes in gastric digests (Chicón, Belloque, Alonso, and López-Fandiño, 2008). As to glycosylated TM, saccharide residues blocked the glycosylation sites of epitopes and reduced the accessibility of TM to trypsin, leading to digests of glycosylated TM with relatively higher allergenicity than the digests of TM.

3.3. Allergy activation of human colon epithelial cells (Caco-2) by TM and glycosylated TM

Caco-2 cells are widely used as model for human colon epithelium cells and commonly used to analyze the biological activity of food allergen towards human intestines. Promotion or inhibition of Caco-2 proliferation provides information about physiological conditions of colon cells.

Compared to the absorbance (OD_{450 nm}) of the PBS activated cells (Fig. 3A), the cell proliferation reduced after challenged by TM and

glycosylated TM, the proliferation suppression changed as follows: TM > TM-M > TM-G > TM-MTS > TM-MPS > TM-MHS, indicating the glycosylated TM had suppression on Caco-2 cell proliferation. Teodorowicz, Fiedorowicz, Kostyra, Wichers, and Kostyra (2013) also found the glucose glycosylated Ara h 1 (peanut 7S globulin) had suppression on Caco-2 proliferation.

Interleukin-8 (IL-8) is a key mediator of epithelial immune responses which influences the activation, growth, differentiation and migration of immune cells including basophils (Eckmann et al., 1993; Rossi & Zlotnik, 2000). Therefore, the secretion of IL-8 by intestinal epithelial cells via glycosylated TM may provide new information in immunomodulating food allergy. As shown in Fig. 3B, compared with the control, the IL-8 release of TM activated cells got increased, and TM > TM-M > TM-G > TM-MTS > TM-MPS > TM-MHS, indicating the glycosylated TM resulted in the increased IL-8 release. As to glycosylated TM, the IL-8 release weakened significantly, which might be due to that saccharide residues masked TM epitopes and blocked the affinity of TM to Caco-2 cell, leading to weaker IL-8 release. Teodorowicz et al. (2013) found that compared with native Ara h 1 (peanut 7S globulin), the glucose glycosylated Ara h 1 (peanut 7S globulin) suppressed the IL-8 release of Caco-2 cells.

3.4. Allergy activation of human basophils (KU812) by TM and glycosylated TM

Basophils are effector cells of allergy reactions and synthesize pro-inflammatory and vasoactive mediators such as histamine. As shown in Fig. 4A, compared to the PBS sensitized basophils, the histamine release had a sharp increase after challenged by TM, while compared with TM, the histamine release of glycosylated TM got weaker: TM > TM-M > TM-G > TM-MTS > TM-MPS > TM-MHS, indicating the glycosylated TM

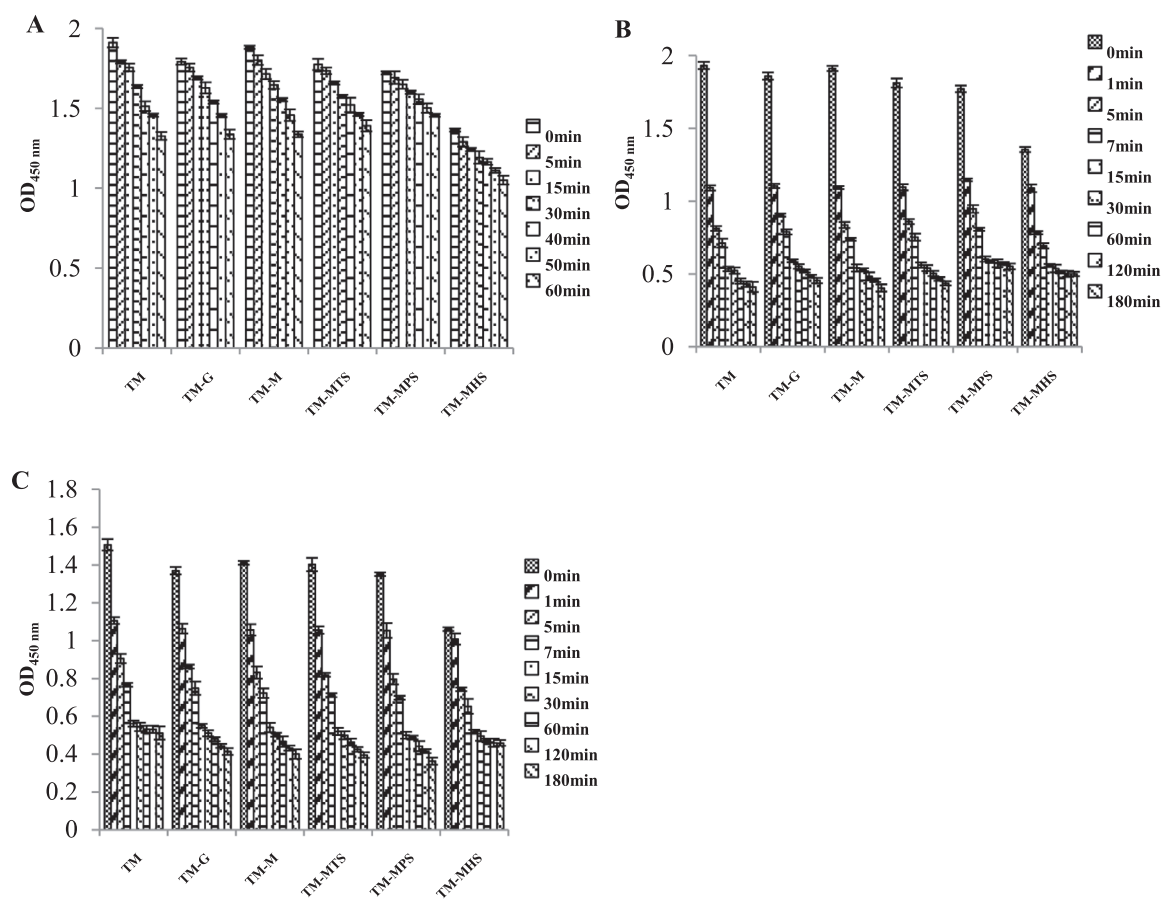


Fig. 2. The allergenicity of simulated gastric digests (A), simulated intestinal digests (B) and simulated gastrointestinal digests (C) of TM and glycosylated TM. Different superscript letters means significant differences between values with different treatment ($p < 0.05$).

exerted stronger basophil activation and more histamine release. This might be due to that: (1) The saccharide residues of glycosylated TM could mask the spatial space of epitopes and block the interactions between IgE and TM epitopes; (2) glycation of TM epitopes could block the accessibility of epitopes to IgE, resulting in weaker basophil activation.

The basophil degranulation was determined by the release of β -hexosaminidase from KU812 basophils. As shown in Fig. 4B, compared

with the control, the release of β -hexosaminidase from basophils activated by glycosylated TM got decreased: TM > TM-M > TM-G > TM-MTS > TM-MPS > TM-MHS, which suggested the glycosylated TM induced weaker basophil degranulation and led to decreased β -hexosaminidase. This might be due to that saccharide residues masked TM epitopes and blocked the affinity of TM to IgE, resulting in weaker basophil degranulation.

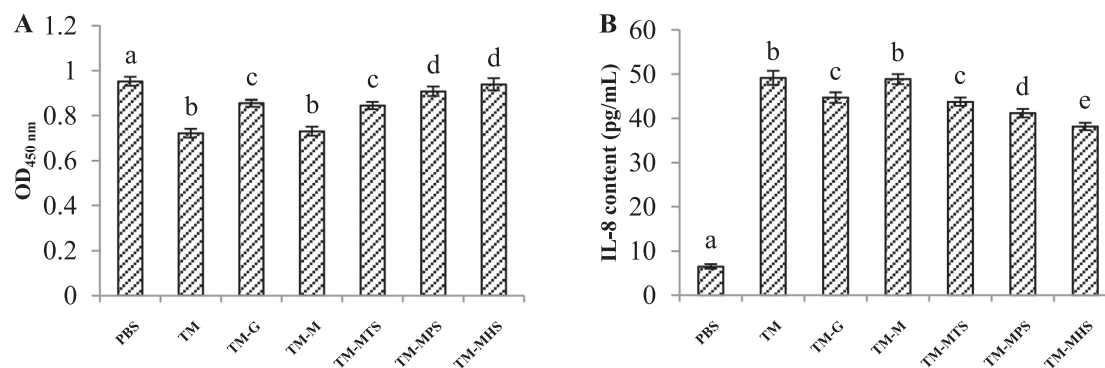


Fig. 3. Effects of TM and glycosylated TM on cell proliferation (A) and IL-8 secretion (B) of Caco-2 cells. Note: Different superscript letters means significant differences between values with different treatment ($p < 0.05$).

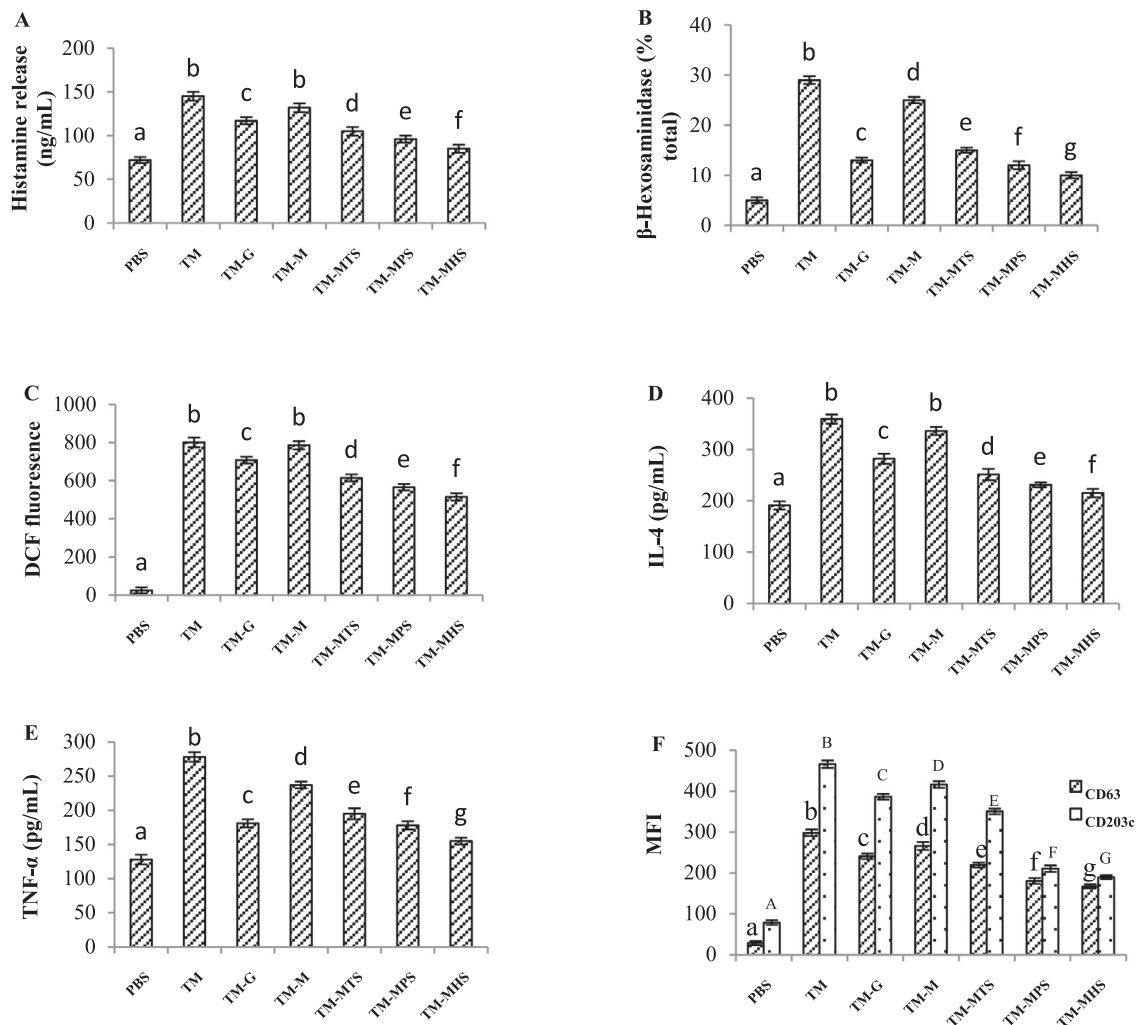


Fig. 4. Effects of TM and glycosylated TM on the histamine release (A), basophil degranulation (B), ROS generation (C), IL-4 secretion (D), TNF- α secretion (E), CD63 and CD203c expression (F) of KU812 basophil cells. Note: Different superscript letters means significant differences between values with different treatment ($p < 0.05$).

Compared with the PBS control, TM and glycosylated TMs had strong allergy activation on the generation of ROS (Fig. 4C), IL-4 secretion (Fig. 4D) and tumor necrosis factor (TNF)- α secretion (Fig. 4E). While compared with TM, the glycosylated TM had weaker activation on secretion of ROS, IL-4 and TNF- α . Specifically, basophil cells release ROS in response to various stimuli, and ROS from basophil cells are involved in allergy inflammation. The result in Fig. 4C showed that TM-G, TM-MTS, TM-MPS and TM-MHS down-regulated the production of intracellular ROS, while TM-M had relatively weaker impact on ROS generation. In addition, basophils also played key role in initiating and perpetuating the inflammatory response in allergy reactions by secreting cytokines (such as IL-4 and TNF- α). The results suggested that the TM-G, TM-MTS, TM-MPS and TM-MHS significantly suppressed the release of IL-4 and TNF- α in basophil cells (Fig. 4D-E), while TM-M had relatively weaker suppression on IL-4 and TNF- α secretion.

Basophil activation was typically associated with the up-regulation of cell surface antigens, CD63 and CD203c belongs to the most important antigens (Kneidinger et al., 2008). Flow cytometry was used to assess the expression of CD63 and CD203c antigens of KU812 cells using the MFI (mean fluorescence intensity). As shown in Fig. 4F, compared with the TM sensitized KU812 basophils, TM-G, TM-MTS, TM-MPS and TM-MHS were found to reduce CD63 and CD203c antigen expression on stimulated KU812 cells, while TM-M had no obvious

regulation on CD63 and CD203c antigen expression. The results might be due to that there was more loss of TM epitopes than the generation of neoallergens during the glycation of TM by MTS, MPS and MHS, inducing the diminished activation of basophils and expression of surface antigens. While during the formation of TM-G and TM-M, the generation of neoallergens (such as N $^{\epsilon}$ -(carboxymethyl)-lysine, CML) might partly counteract the loss of TM epitopes, resulted in the relatively lower changes on surface antigen expression.

To gain insight into how saccharides with different molecular weight regulated the activation signaling of basophils, the phosphorylation of MAPKs was examined. The activation of tyrosine kinases initiated an intracellular signaling cascade involving activation of MAPKs. The MAPK play an important role in anaphylactic reaction in humans (Qi & Ellison, 2005). It has been shown that sequential allergen desensitization of basophils involves the phosphorylation of MAPK and is correlated with down-regulation of CD63 and CD203c antigens (Christensen et al., 2014). In this work, TM-G, TM-M, TM-MTS, TM-MPS and TM-MHS were able to inhibit the phosphorylation of MAPKs. Due to the suppression of these signaling pathway molecules, glycation was shown to be able to counteract even suppress the activation of basophils. As shown in Fig. 5, kinases of signaling molecules were determined using Proteome Profiler Array, the expression of MAPKs in the glycosylated TM groups were significantly lower than those of the TM

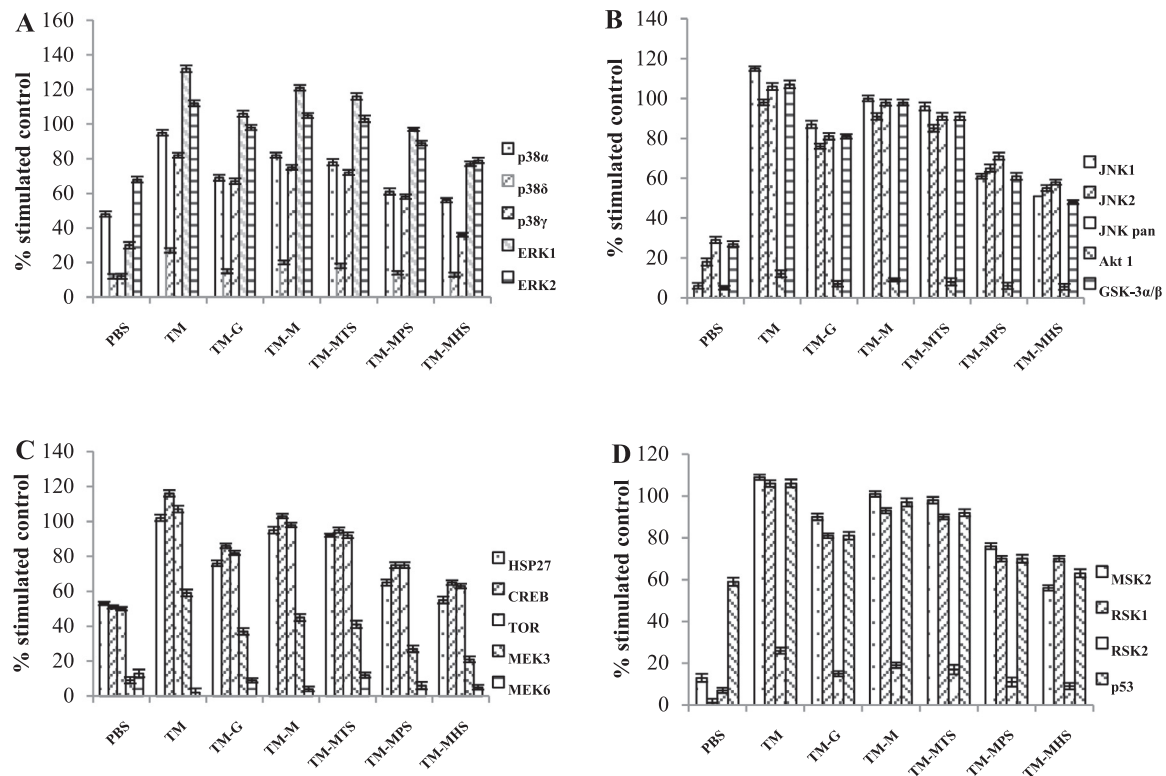


Fig. 5. Effects of TM and glycated TM on MAPK signaling of KU812 basophils.

activated group. Meanwhile, compared to the basophils stimulated by TM, the phosphorylation level of MAPK was suppressed by TM-G, TM-MTS, TM-MPS and TM-MHS, caused a large reduction and suppressed the phosphorylation level of MAPKs. While compared with the basophils stimulated by TM, the TM-M had relatively weaker modulation on the phosphorylation of MAPKs, which might be due to the generation of AGEs neoallergens (such as CML) counteracted the destroying/masking of TM epitopes by saccharide residues.

In summary, compared with TM, the TM-G, TM-MTS, TM-MPS and TM-MHS could suppress allergy activation of basophil cells, while TM-M had insignificant allergy activation of basophil cells when compared with TM-MTS, TM-MPS and TM-MHS. During glycation, the TM epitopes that containing potential glycation sites (lysine, arginine and histidine) were destroyed, while there may be AGEs neoallergens (such as CML) generated at the same time. The RAGE (receptor for advance glycation and products) was a *trans*-membrane protein that acted as an important factor in the induction of immunological response induced by AGEs (Hofmann et al., 1999; Miyata et al., 1996; Schmidt et al., 1994). AGEs could be recognized by the RAGE on basophil cells (Han et al., 2011; Gupta et al., 2018), which functioned as potent epitopes to strengthen allergenicity. As to TM-G, TM-MTS, TM-MPS and TM-MHS, the epitopes loss may be greater than the generation of neoallergens, thereby inducing the lower allergenicity. While as to TM-M, the generation of neoallergens might partly counteract the TM epitope loss.

4. Conclusions

In conclusion, glycation of TM by glucose, MTS, MPS and MHS (TM-G, TM-MTS, TM-MPS and TM-MHS) could significantly destruct and mask TM epitopes to obtain lower allergenicity, while glycation of TM by maltose (TM-M) had insignificant suppression on TM allergenicity. In addition, TM-G, TM-MTS, TM-MPS and TM-MHS inhibited the proliferation and IL-8 secretion of Caco-2, and the CD63 and CD203c expression, MAPK signaling of KU812 basophils, while TM-M had

insignificant suppression on the allergic relativities of Caco-2 and KU812 cells. Glycation of TM by saccharides with larger molecular sizes (such as MHS) could provide new insight into the desensitization of shrimp-induced food allergy, while glycation of TM by maltose is not suitable for desensitize shrimp-induced food allergy.

Declaration of interest statement

None declared.

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