

FULL ARTICLE

The IgE/IgG binding capacity and structural changes of Alaska Pollock parvalbumin glycated with different reducing sugars

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Abstract

Parvalbumin (PV) is one of the major allergens in fish. The aim of our present work was to research the influence mechanism of glycation with different reducing sugars (glucose, fructose, ribose, lactose, and galactose) on the immunoglobulin E (IgE) and immunoglobulin G (IgG) binding capacity and structure changes of PV in Alaska Pollock. PV glycated with glucose or fructose (PV-Glu/ PV-Fru) exhibited the higher IgE/IgG binding capacities than that of ribose, galactose, or lactose. During glycation, the lysine (Lys), tyrosine (Tyr), and phenylalanine (Phe) of PV were gradually embed into core area of three-dimensional structure of protein, which reflected in the ultraviolet (UV) spectrum and fluorescence spectra. Moreover, the increase of surface hydrophobicity had confirmed the conformation alteration of glycated PV. These results suggest that there is a specific association among the change of PV in glycation and in potential allergenicity. The types and conformation of reducing sugar greatly influenced the IgE/IgG binding capacity of PV, and glycation with ribose and galactose was a promising approach for reducing the IgE/IgG binding capacity of PV from Alaska Pollock.

Practical applications

Parvalbumin (PV), the major allergen of fish, it can not only maintain the physiological activity of cells, but also cross react with human amyloid protein to alleviate Alzheimer's disease and Parkinson's syndrome. This study revealed that the IgE/IgG binding capacity and structural changes of PV from Alaska Pollock modified by glycation with different reducing sugars. This will help us to understand the sensitization and structural change of the glycated products after the reaction of PV with different reducing sugars. It provides an effective carbonyl source for the preparation of low antigenicity PV based on glycation and lays a foundation for glycation modification of other food allergens.

KEYWORDS

Alaska Pollock, glycation, IgE/IgG binding capacity, parvalbumin, structural changes

Abbreviations: ELISA, indirect competition enzyme-linked immune sorbent assay; Fru, fructose; Gal, galactose; Glu, glucose; IgE, immunoglobulin E; Lac, lactose; Phe, phenylalanine; PV, parvalbumin; Rib, ribose; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; Trp, tryptophan; Tyr, tyrosine; UV, ultraviolet.

1 | INTRODUCTION

Fish consumption has grown enormously over the past several decades, making it to be the main way for body to obtain high-quality protein. However, fish proteins, together with other allergens, including egg and milk proteins, are a frequent cause of food allergies. About 0.1% of children and 1.1% of adults have the allergic reaction to fish in United States (Sharp & Lopata, 2014). In Morocco, the prevalence of food allergy in children (28.2%) was higher than that in adults (16.8%) (Najlae Mejrhit, 2018). The major clinical symptoms of fish allergy are urticaria, asthma, and diarrhea, which sometimes even will be life-threatening. For fish allergic patients, the most useful method is to avoid touching the allergens completely, but this will inevitably lead to the lack of nutrients. In addition, the fish allergic compounds are widely existed in food products, and the allergic reaction is not only caused by the gastrointestinal digestion and absorption, but also by the inhalation from the respiratory tract. Therefore, it is necessary to develop an effective method to reduce the antigenicity of fish products.

The major allergen of fish, Gad c I, which can cause 90% fish allergic reaction, and it was first identified from cod (*Gadus callarias*) (Johansson, 1972). Because of the characteristics of strong solubility and low molecular weight (10.5–12.2 kDa), the protein was called parvalbumin (PV) (Pechère, 1968). PV is widely distributed in the sarcoplasmic proteins of fish, it is an acidic protein (pI: 3.9–5.5), and has a high affinity for Ca^{2+} (two Ca^{2+} per molecule) (Arif et al., 2006). As an intracellular protein, PV can promote muscle contraction through the calcium conduction, and the content of it in the white muscles of lower vertebrates is higher than that in dark muscles. PV is often subdivided into two subtypes, α -PV and β -PV, which contains 108 and 109 amino acid residues, respectively (Li et al., 2014). However, most studies on cod allergens were focused on Atlantic cod (Perez-Gordo et al., 2013) and Baltic cod (Minkiewicz et al., 2012), and less about Alaska Pollock. Alaskan Pollock is rich in high protein, and have the tender meat, which can improve the body's immunity and memory, and is popular with consumers. In the United States, the production accounts of Alaskan Pollock for more than a third of the total fish, and it is mainly used for the production of cod fillet, surimi and fish fillet, some of them are processed into cod fillet and cod fillet by KFC, McDonald's and other chain restaurants, and the other are sold directly as frozen fish fillets (Zhou et al., 2006). In addition, there are two amino acid sequences of β -PV in Alaska Pollock. Thus, this paper selected Alaska Pollock as the object to study the method of reducing antigenicity. At present, the common modification methods of food allergens are heating (Zhou et al., 2006), phosphorylation (Jockusch et al., 1988), glycation (de Jongh et al., 2013), and biological recombination (Swoboda et al., 2002). Nevertheless, the low antigenicity PV prepared by bioengineering and phosphorylation modification not only has not been proved to be safe, but also has many limitations in fish processing. Consequently, glycation has been considered as a safe and satisfactory way to improve the antigenicity of food allergens. Glycation is the first step of the Maillard reaction, which happens between protein and reducing

sugars. This nonenzymatic reaction plays an important role in food processing, and it also has a great contribution to improve the flavor and functional properties of food. Recently, glycation has been used to modify potential antigenicity of PV by the covalent binding with reducing sugar. de Jongh et al. (2013) found that PV showed the low IgE-binding capacity after glycated modification. Zhao et al. (2017) found that the IgG/IgE of recombinant PV decreased significantly after glycation. However, there are many factors to influence the glycation reaction, and the type of reducing sugar is one of the most important factors in glycation. Ribose exhibits the fastest rate in glycation, it is often used as the raw material of ATP, NADH, and other biochemical metabolism molecules; glucose is the main substance that provides energy in biological organisms, and fructose is the isomer of glucose; lactose is a unique carbohydrate in mammal milk, including one molecule of glucose and one molecule of galactose, and it can not only provide energy, but also promote brain development. Moreover, there are few studies on the antigenicity and structural changes of PV during glycation, thus, to study the IgE/IgG binding capacity and structural changes of PV could provide the theoretical basis for the further application of glycation in fish allergen.

In this work, in order to estimate the changes of IgE/IgG binding capacity of PV after glycation with different reducing sugars, as well as to expound upon the relationships between them, PV was glycated with glucose (Glu), fructose (Fru), ribose (Rib), lactose (Lac), galactose (Gal) under dry-heating condition. The structural changes were characterized through the content of free amino acids, UV absorption spectrum, intrinsic and synchronous fluorescence spectra, and H_0 . The IgE/IgG binding abilities were determined by indirect competition ELISA.

2 | MATERIAL AND METHODS

2.1 | Materials and reagents

D-(+)-galactose, D-(+)-lactose, D-(+)-ribose, D-(+)-fructose, and D-(+)-glucose were purchased from Sigma-Aldrich (St. Louis, MO, USA). Frozen pieces of Alaska Pollock were purchased from Hongyuan aquatic food company (Beijing, China). The polyclonal antisera against PV obtained from four young (3 months old, about 2.0 kg) Japanese male rabbits. Human sera numbered P1–P4, whose specific IgE levels were 24.8, ≥ 100 , 35 and 33.5 kU/L (measured by immune CAP), were brought from PlasmaLab International (Everett, WA, USA) and stores at -80°C until used. The detailed information of four fish allergen patients is shown in Table 1. Goat antihuman IgE-HRP conjugate (A9667-2 MI) and goat anti-rabbit IgG-HRP conjugate (SE101-1 ml) were from Immunology Consultants Laboratory, Inc. (Oregon, Portland). 8-anilino-1-naphthalenesulfonate (ANS), 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), Freund's adjuvant, and 3,3',5,5'-tetramethylbenzidine (TMB) were purchased from Sigma-Aldrich (St. Louis, Mo., USA). All other reagents were analytical grade. Ultrapure water from water purification system (Millipore, Bedford, MA, USA) was used in this study.

TABLE 1 The medical history of fish allergy (FA) patients

Number	Age	Sex	Symptoms	The IgE level (kU/L)
P1	35	Female	AR; EC; AS. Started at 15	24.8
P2	30	Female	AR; EC; AS. Started at 6	≥100
P3	54	Male	AR; EC; AS. Started at infant	35
P4	51	Male	AR; EC; AS; UT. Started at infant	33.5

Abbreviations: AR, allergic rhinitis; AS, asthma; EC, eczema; UT, urticarial.

2.2 | Extraction and purification of PV

The extraction and purification was used the method of Li et al. (2014) and made some modifications. The frozen pieces Alaska Pollock were unfrozen, mashed, and homogenized with four volumes of 20 mM Tris-HCl (pH 7.5) and stood for 24 hr. The homogeneous solution was filtered, the supernatant was incubated in a water bath at 95°C for 30 min. After centrifugation at 8,000× *g* for 25 min, the supernatant was added with ammonium sulfate to the saturation of 60%, settled for 8 hr at 4°C, and centrifuged for 20 min at 4°C. Then, the supernatant was collected and added with ammonium sulfate to the saturation of 80%, and repeated the step of settling and centrifuging. After them, the supernatant was centrifuged and added with ammonium sulfate to 100%, then, settled and centrifuged. The precipitate was dissolved in the Tris-HCl (20 mM, pH 7.5), and the remaining salt was removed by Millipore ultrafiltration (molecular weight of the intercepted is 3,000 Da).

2.3 | MALDI-TOF analysis

Molecular weight (MW) of the purified PV was analyzed using 4800 MALDI-TOF-TOF mass spectrometer (AB Science, USA) according to a previously reported method (Zhang et al., 2014). Matrix was prepared by dissolving 10.0 mg/ml of sinapic acid in 50% acetonitrile with 50% of Tris-HCl (10 mM pH 7.0) buffer solution. The proteins were diluted into 1:100 with ultrapure water. 1.5 µl of mixtures at 1:1 protein solution to matrix ratio was spotted onto MALDI target, then, flowed by air-drying, then, the measurement and analysis were carried out. Each sample is located in at least three different positions on the plate, and the scanning range of each point is 0 ~ 100 kDa.

2.4 | PV Samples Preparation

PV solutions (1 mg/ml, dissolved in distilled water) were placed in centrifuge tubes, then, the different reducing sugars (glucose, fructose, ribose, lactose, and galactose) were added into the solutions (the mass ratio of PV and sugars were 1:1). After mixing, all the

samples were lyophilized and incubated at 60°C and 65% relative humidity (saturated sodium chloride solution) for 1 hr. After incubation, the untreated sugars were removed by Millipore ultrafiltration (molecular weight cut off 3,000 Da). Finally, all the samples were lyophilized, and stored at -20°C. Native PV was called PV. Heated PV was recorded as PV-H, and the glycated samples were named PV-Glu, PV-Fru, PV-Rib, PV-Lac, and PV-Gal, respectively.

2.5 | IgE/IgG binding abilities determination

The IgE/IgG binding capacity of PV samples was estimated by indirect competition ELISA with human antisera and rabbit antisera (Taheri-Kafrani et al., 2009). First, the 96-well microplate was coated 1 hr at 37°C with PV (100 µl per well, 4 µg/ml). After washing with PBST (0.05% Tween-20 in 50 mmol/L, pH 7.4), the microplate was blocked with 2% of BSA for 2 hr at 37°C, and washed again. Then, 50 µl of anti-sera samples (1:500 diluted human sera or 1:10,000 diluted rabbit sera) and 50 µl of PV samples (inhibitors, 4 µg/ml) were added after washing. After incubation, remove the solution and wash the plate. A 100 µl of goat antihuman IgE-HRP conjugate or goat anti-rabbit IgG conjugate (1:800 diluted IgE conjugate or 1:5,000 diluted IgG conjugate) were added and incubated for 1 hr at 37°C. After washing, 100 µl of TMB solution was added and incubated for 15 min at 37°C. Finally, 50 µl of 2 mol/L sulfuric acid was added to stop the reaction and the absorbance was measured at 630/450 nm by the microplate reader (HF2000, Huaan Magnech, Beijing, China). The inhibition rate was calculated: Inhibition (%) = $(1 - B/B_0) \times 100\%$, where B and B₀ are the absorbance values of the well with and without glycation samples, respectively. Each sample was performed in triplicates.

2.6 | Determination of free amino groups

The degree of glycation was evaluated by measuring the free amino groups using the o-phthaldialdehyde (OPA) method (Nielsen et al., 2001). Freshly prepared OPA reagent was fresh for using which was mixed with 100 µl of β-mercaptoethanol, 25 ml of 0.1 M sodium tetraborate solution, 2.5 ml of 20% sodium dodecyl sulfate (SDS), and 4 mg OPA, respectively, which was dissolved in 1 ml of methanol. The final volume was adjusted to 50 ml with distilled water. The samples containing 50 µg of protein were mixed with the OPA reagent, and incubated for 2 min at 37°C. Absorption was measured at 340 nm against a blank containing the OPA reagent. Working standards were prepared by serial lysine dilutions to final concentrations from 0 to 1 mg/ml. Three repetitions were performed for each sample.

2.7 | UV absorption spectroscopy

The ultraviolet (UV) absorbance spectra were recorded on a UV array spectrophotometer (UV-2910, Hitachi, Tokyo, Japan) using a

quartz cuvette with a concentration of 1 mg/ml in ultrapure water. The UV absorption spectra were recorded and scanned from 240 nm to 400 nm.

2.8 | Fluorescence spectrum

The intrinsic fluorescence determination and synchronous fluorescence analysis were obtained in using a fluorophotometer (F-4500; Hitachi, Tokyo, Japan). Protein concentration of PV was 1 mg/ml, the excitation wavelength was 295 nm, the emission spectrum was recorded from 300 nm to 500 nm, and the slit and scan speed is 5 nm and 1,200 nm/min, respectively. The synchronous fluorescence spectra were recorded within the scanning ranges, and the wavelength interval was fixed individually at $\Delta\lambda = 15$ nm and 60 nm.

2.9 | Surface hydrophobicity

1-Anilinonaphthalene-8-sulfonate (ANS) was fluorescent when it is associated with hydrophobic residues, and thus, it is used to determine hydrophobic domains in proteins (Nielsen et al., 2001). In the present work, samples were diluted to a gradient (0.025–0.4 mg/ml) using phosphate-buffered saline (PBS, 10 mM, pH 7.4), respectively. Then, 4 ml of the samples were mixed with 20 μ l bis-ANS with 8 μ M. Fluorescence emission scan was obtained from 400 nm to 600 nm, with excitation at 370 nm using a Hitachi fluorophotometer (F-7000, Hitachi, Tokyo, Japan). The surface hydrophobicity of the sample was defined as the ratio of the fluorescence absorption to different concentrations.

2.10 | Statistical analysis

All experiments were carried out in triplicate, and all results were presents mean value $\pm$ standard deviation (SD). Original Pro 2017 (original lab Corp, USA) was used for data curve fitting, and GraphPad prism 8 (GraphPad Software, San Diego, CA, USA) was used for mapping.

3 | RESULTS AND DISCUSSION

3.1 | Extraction and purification of PV

In this study, SDS-PAGE and MALDI-TOF were adopted to evaluate the changes of molecular weight during the process of the extraction and purification of PV, which are shown in Figure 1. As shown in Figure 1a, line 1 was the mycoplasma protein from Alaska Pollock, there were many bands, the molecular weight ranges from 11 to 170 kDa, including enolase subtype (47 kDa), phospho aldehyde dehydrogenase (41 kDa), and PV (12 kDa), which could cause fish allergy, and myoglobin (16.7 kDa), myosin (51 kDa), etc. Line 2 was the supernatant of sarcoplasmic protein after boiling and centrifugation. There were three bands with the molecular weight from 12 to 36 kDa, among them, the 18 kDa may be a new allergen formed during heat treatment, 35 kDa may be a polymer formed by heating PV, while 12 kDa could be our target protein. In line 3, there was single band with a molecular weight of 11–12 kDa, which was consistent with the characteristics of PV. Then, the purity of PV was determined by Image J software was 91.65%, which indicated that this method is more effective to extract and purify PV. And the result of

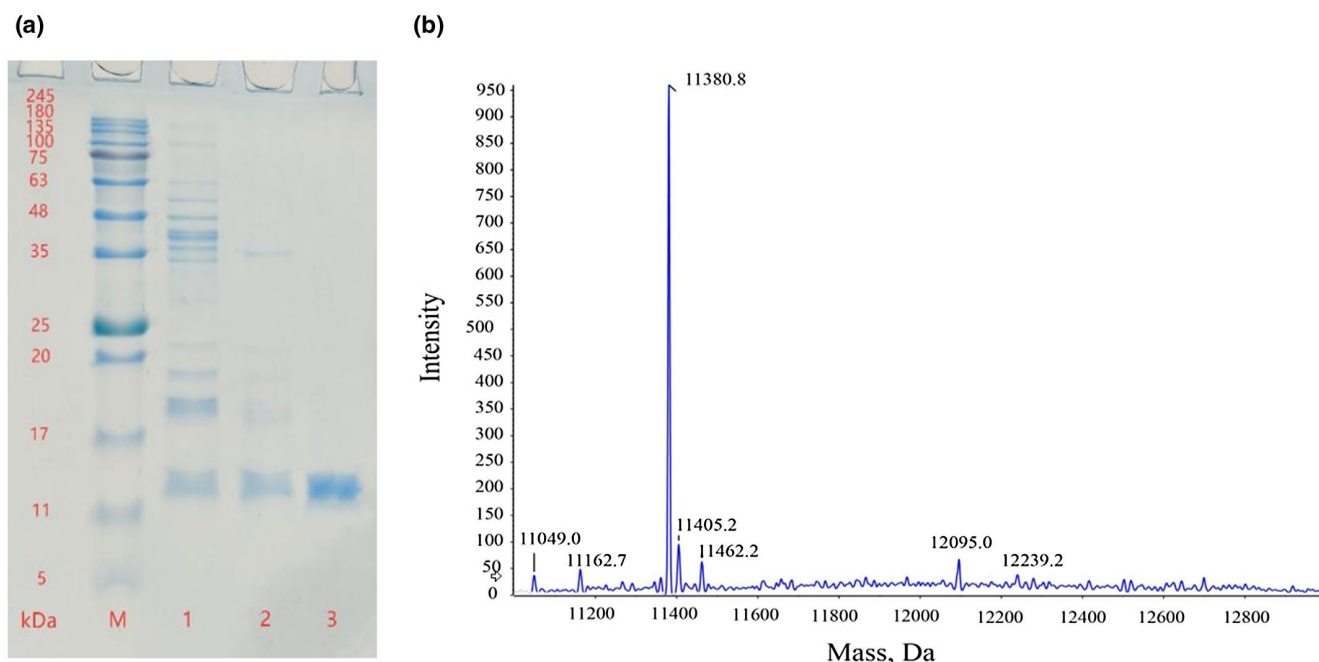


FIGURE 1 The extraction of the parvalbumin (PV) of Alaska Pollock. (a: the SDS-PAGE of PV; b: the MALDI-TOF analysis of PV)

MALDI-TOF is shown in Figure 1b. The PV which prepared by ammonium sulfate precipitation method had showed seven similar molecular weights, they were 11,380.8 Da, 11,405.2 Da, 11,462.2 Da, 11,049.0 Da, 11,162.7 Da, 12,095.0 Da, and 12,239.2 Da, respectively. All of them accord with the molecular weight characteristics of PV. Among them, the abundance of 11,380.8 Da was high, which is the target protein for our experiment, 11,462 Da had the same molecular weight as Atlantic cod (Ma et al., 2008). It had also been reported that Alaska Pollock has similar molecular weight subtypes, indicating that the three proteins were all the subtypes of Alaska Pollock (Perez-Gordo et al., 2011). This result is consistent with the PV obtained by previous column purification (Do et al., 2005), which indicates that a high-purity PV was obtained in this experiment.

3.2 | IgE/IgG-binding ability analysis

The IgE/IgG-binding abilities of PV, PV-H, PV-Glu, PV-Fru, PV-Rib, PV-Lac, and PV-Gal were determined with indirect competitive ELISA with human antisera and rabbit antisera. The binding capacity of antibody to PV was reflected by inhibition, and lower inhibition reveals lower binding capacity. The results are listed in Figure 2. Compared with PV, PV-H exhibited the same inhibition ($p > .05$), PV-Glu and PV-Fru were showed the high inhibitions, whereas the PV-Rib, PV-Gal, and PV-Lac exhibited lower inhibitions, with the lowest value was displayed by PV-Rib. The similar results were observed in the antisera in different fish allergy patients (Figure 2a), and rabbit antisera (Figure 2b). These results indicated that the heating could enable PV to polymerize and destroy some conformational epitopes. After glycation, PV had a covalent binding with different reducing sugars, and glycation could affect the specific recognition of antigen and antibody by changing the conformation of protein, thus, the IgE/IgG binding capacity was changed. Meanwhile, the change of IgE/IgG binding capacity is related to the type of reducing sugars.

Ribose as the most active pentose in glycation, followed by were galactose, glucose, fructose, and lactose. Although the glycation reactivity between lactose and PV was slower than others, the PV-Lac showed the lower inhibition, this result may not because the glycation reaction, but the conversion of lactose to lactulose during the heating, which will participate in the immune response to human IgE serum (Lier & Wd, 1981). Lactulose is a new functional oligosaccharide with good stability, which is not involved in glycation reaction and it is widely used in infant products, beverages, health care, and medical supplies. Interestingly, PV-Glu and PV-Fru shown the high inhibition, the same result was found in the glycation of codfish PV as illustrated by Western blot (de Jongh et al., 2013). In addition, the same conclusion had been found in studies of peanut allergens. Maleki et al. (2000) exhibited the increased IgE-binding capacity of PV-Fru and PV-Glu conjugates of glycated peanut allergens, while Blanc, et al. (2011) reported the decrease effect on the IgE-binding capacity of Ara hr 1 after glycation. We think these results could be attributed to the different glycation conditions, including protein concentration, types and structures of reducing sugars, glycation temperature and time. To show the relationship between the IgE/IgG binding capacity and structural changes of PV by glycated, the structural were measured in subsequent experiments.

3.3 | Free amino groups and UV absorption

The glycated modification might occur preferentially on the protein surface or specific glycation "hot pots," then, decreased the content of free amino groups (Indurthi et al., 2012). The free amino groups content of PV, PV-H, PV-Glu, PV-Fru, PV-Rib, PV-Lac, and PV-Gal are presented in Figure 3a. The free amino groups content of PV-Glu, PV-Fru, PV-Rib, and PV-Gal were lower than that of PV, PV-H, and PV-Lac ($p < .05$), and there was no significant difference between PV-H and PV-Lac ($p > .05$). The lowest content of free amino

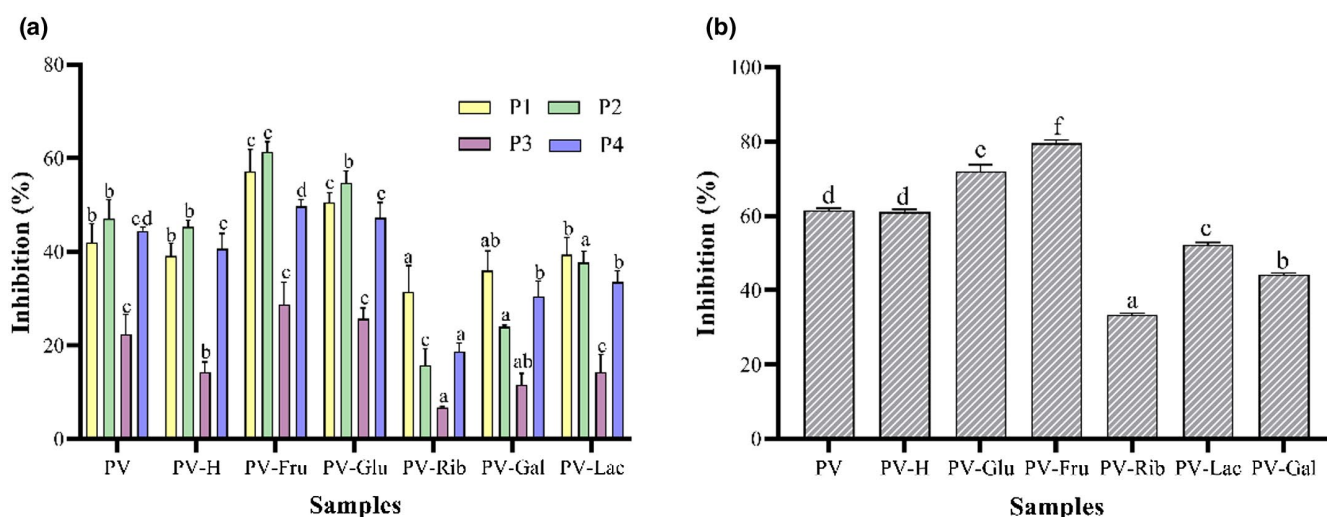


FIGURE 2 Effects of different reducing sugar treatments on the IgE (a) and IgG (b) binding capacity of PV (P1-P4 are the number of FA patients)

groups was found in PV-Rib, and followed by were PV-Gal, PV-Glu, and PV-Fru. This may be related to the activity and rate of glycation reaction between different reducing sugars and PV. Ribose has the short carbon chain of ribose. In aqueous solution, pentose not only has a higher chain structure than hexose, but also has a higher ring structure. Simultaneously, Glucose and galactose is aldose, fructose is ketose. The electrophilic activity of aldose is higher than that of ketose, and the terminal group has smaller steric hindrance, which makes the amino compounds are easier to approach (Jing & Kitts, 2004). The similar conclusion was drawn in our previous study on the α -lactalbumin ultrasonic pretreatment combined dry-state glycation (Liu, Tu, Liu, et al., 2018). As expected, the modification degree was direct relation between the sugar size and structure, they were PV-Rib, PV-Gal, PV-Glu, PV-Fru, and PV-Lac, respectively. This result was consisted with the research, which were found in β -Ig and thiamine systems with different reducing sugar systems, respectively (Chevalier et al., 2001).

Subsequently, the UV absorption spectra were used to determine conformational change through the new chromophores and absorption intensity, which is shown in Figure 3b. Due to the high content of Phe in PV, the maximum absorption peak of PV was at 258 nm, which was consistent with previous reports (Do et al., 2005). Compared to PV, the UV absorption of glycated samples significantly decreased, but there were not significant changes. In addition, PV-Fru, PV-Rib, PV-Lac, and PV-Gal had a slight red shift. These results indicated the glycation could change the spatial structure of aromatic amino acid residues and significantly changed the conformation of PV. Especially for the chromogenic groups, such as tyrosine (Tyr), tryptophan (Trp), and phenylalanine (Phe), which were moved to the interior of protein molecule when the protein was glycated. When the protein was glycated, these chromophores will migrate to the interior of the protein molecule and show a low UV absorption.

3.4 | Intrinsic fluorescence spectra and Surface hydrophobicity (H_0)

The effects of different reducing sugar treatments on intrinsic fluorescence and surface hydrophobicity (H_0) of PV are shown in Figure 4. The intrinsic fluorescence is mainly contributed of tryptophan (Trp), tyrosine (Tyr), and phenylalanine (Phe) residues, and the maximum emission wavelengths are 348, 303, and 282 nm, respectively. Therefore, the change of protein conformation can be described by intrinsic fluorescence, Figure 4a showed the fluorescence emission spectra of Tyr in PV, PV-Glu, PV-Fru, PV-Rib, PV-Lac, and PV-Gal. When excited at 295 nm, the PV exhibited a fluorescence emission maximum (λ_{max}) at 300 nm. The intensity of Tyr-related fluorescence was decreased after glycation, this implied that the conformational structure had changed by glycation modification, including covering up of the glycated PV-conjugates occurred around the Tyr residues. The same result was also found in our previous study that the ultrasonic pretreatment combined with glycation (Liu et al., 2017), due to the relatively increased exposure of Tyr and Trp residues in more hydrophilic surroundings, solvent relaxation, and more quenching agents were produced in the conjugates. Interestingly, the intrinsic fluorescence intensity of PV-Glu was higher than PV-H, suggesting that the access of glucose might stabilize the structure of the PV to avoid the excessive damage of heat treatment (Delgado et al., 2014). Nevertheless, the fluorescence intensity of PV-Lac was lower than PV-Glu, it may result from PV-Lac was in the intermediate period of Maillard reaction. The Amadori compounds have no fluorescence properties in the primary stage, but the protein can be cross-linked with adjacent proteins or amino acids, and produced polymers with fluorescence characteristics in the prophase of Maillard reaction. In addition, in the glycation reaction, the fluorescence intensity of the product is reduced, because of

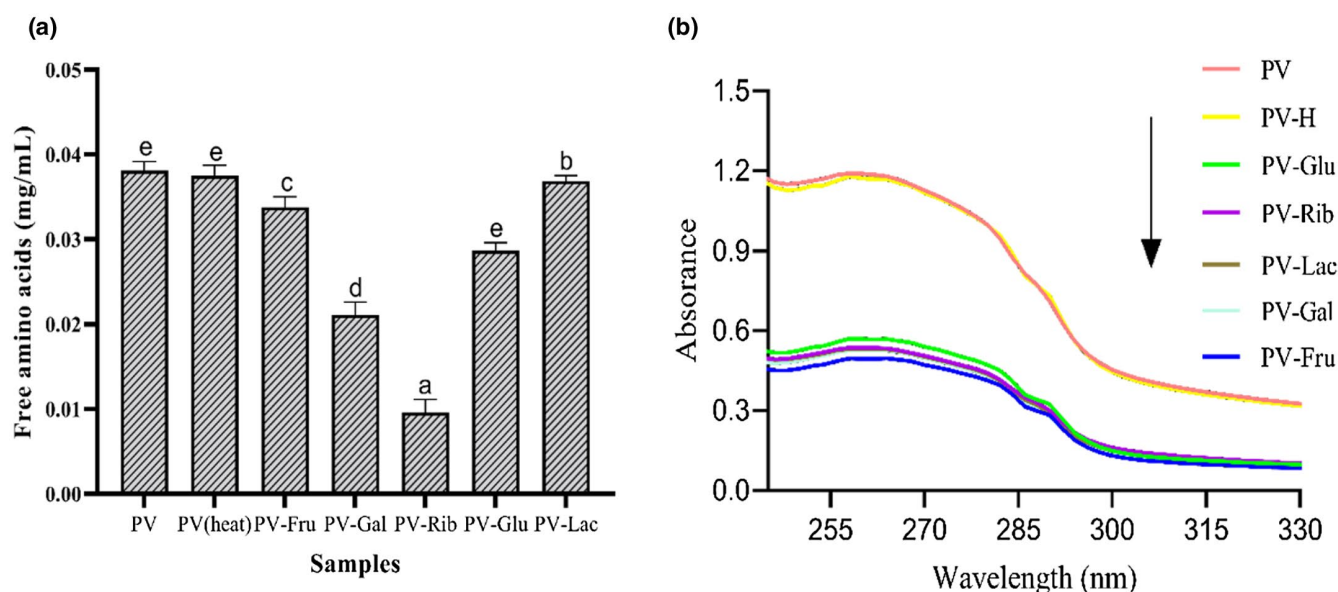


FIGURE 3 The effect of free amino acid (a) and ultraviolet absorption spectrum of PV-conjugates (b)

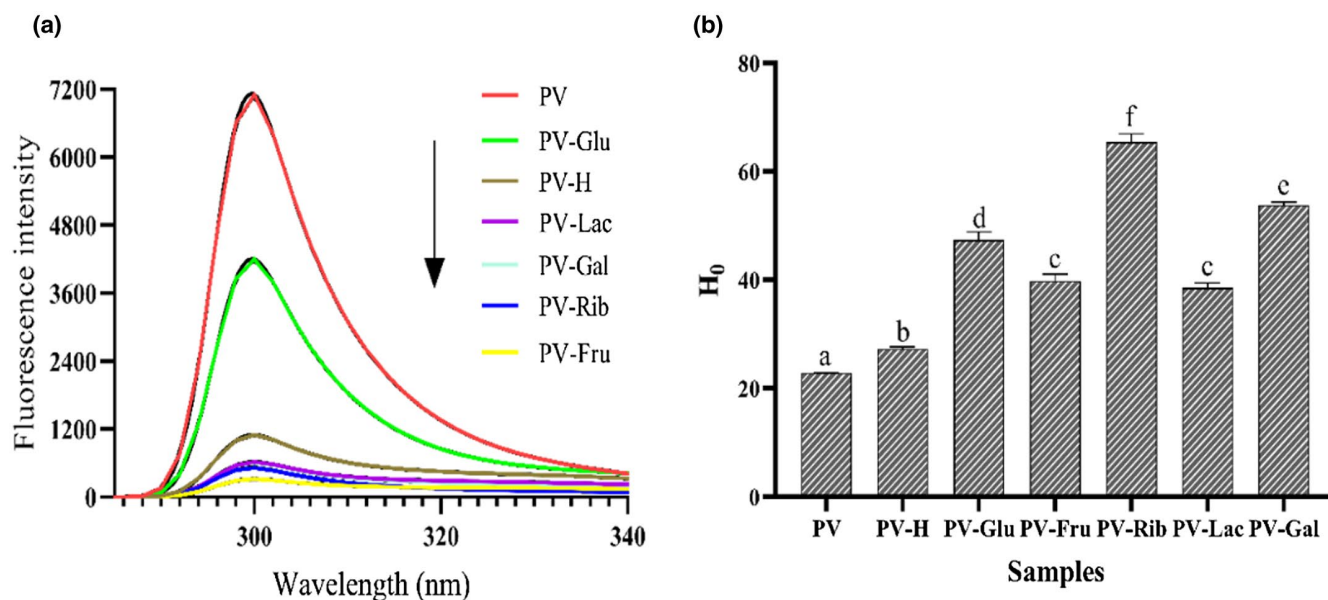


FIGURE 4 Effects of different reducing sugar treatments on intrinsic fluorescence (a) and surface hydrophobicity (b) of PV

the blocked Tyr region in PV, which shows that the glycation reaction can significantly change the structure of PV. With the different kinds of reducing sugars, the structure changes differently, this result was consistent with UV absorption.

Based on this, this article had described the H_0 of PV, and the result is shown in Figure 4b. H_0 is the parameter of hydrophobic groups on the surface of protein in contact with polar aqueous solution, which is one of the main forces to maintain the tertiary structure of protein and is often used to evaluate the spatial structure of protein (Zhang et al., 2012). Compared with PV, the H_0 of PV-Glu, PV-Fru, PV-Rib, PV-Lac, and PV-Gal had increased ($p < .05$), and there was no significant difference between PV-Fru and PV-Lac ($p > .05$), which indicated that the conformation of PV was changed after heat treatment and glycation, and some hydrophobic groups and regions were exposed. Among the PV-conjugates, PV-Rib had the highest H_0 , followed by were PV-Gal, PV-Glu, PV-Fru, and PV-Lac, this result was

consistent with the results of IgE/IgG binding capacity, fluorescence, and free amino content, all of them also shown that the structure of reducing sugar has a greater impact on the product in glycation.

3.5 | Synchronous fluorescence spectra

Synchronous fluorescence spectroscopy is an effective method for analyzing the microenvironment of amino acid residues, which involves measuring the emission wavelength shift. The simultaneous scanning of the excitation and emission of monochromators was set with a wavelength difference ($\Delta\lambda = \lambda_{em} - \lambda_{ex}$) of 15 nm and 60 nm, respectively. As showed in the Figure 5, the synchronous fluorescence of PV, PV-H, PV-Glu, PV-Fru, PV-Rib, PV-Gal, and PV-Lac provided the characteristic information of the Tyr (a) and the Trp (b) residues. Compared with PV, the synchronous fluorescence

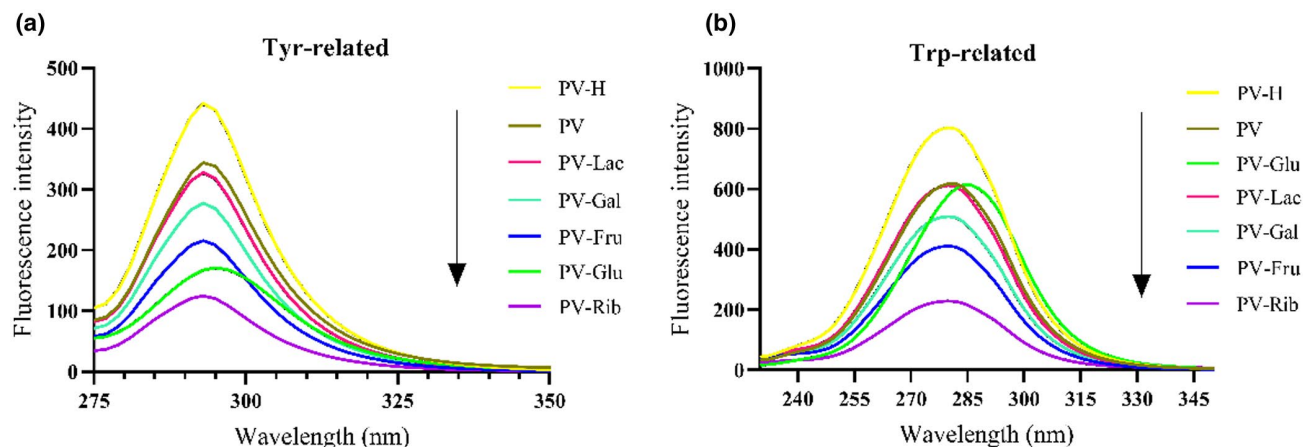


FIGURE 5 Effects of different reducing sugar treatments on synchronous fluorescence of PV (a: The characteristic of Tyr residues in PV after glycation; b: The characteristic of Trp residues in PV after glycation)

intensity of PV-Fru, PV-Rib, PV-Gal, and PV-Lac decreased without any remarkable wavelength shift. Contrarily, the fluorescence intensity of PV-H was higher than that of PV, and the maximum excitation wavelength of PV-Glu had a red shift. Simultaneously, there had an energy transfer from Tyr to Trp residues in PV-Lac, PV-Gal, PV-Fru, and PV-Glu when after glycosylated modification, and caused the fluorescence quenching of Tyr residues and fluorescence enhancement of Trp residues (Kalapathy et al., 1997). The process of unfolding and exposed more Tyr and Trp residues of PV undergo a heat treatment. The change of maximum excitation wavelength in PV-Glu displayed that the glycosylated modification increased the molecular extensibility of PV. At the same time, it was further explained that the binding site of PV-Glu is closer to Trp, and the hydrophobicity of PV-Glu was decreased (Wang et al., 2007).

3.6 | The conformational changes of Alaska Pollock PV induced the reduction in IgE/IgG binding capacity

Glycosylated modification can significantly affect the conformational structure of PV, which was closely related to the IgE/IgG binding capacity. In order to explore the reason about this, we investigated the change of conformational structure and IgE/IgG binding capacity of PV-conjugates between PV and different kinds of sugars. The results showed that the structural changes were responsible for reducing the IgE/IgG binding capacity of PV. As we all know, the conformation epitopes play an important role in the food allergy. When PV was treated with glucose, fructose, ribose, galactose, and lactose, the structure of them had changed significantly, but IgE/IgG binding capacity had a great difference. The glycosylation induced the unfolding PV, which reflecting in the decrease in the intrinsic and synchronous fluorescence. PV-Rib and PV-Gal promoted the decrease of IgE/IgG-binding capacity compared with PV and PV-H, the glycosylated modification resulted in the masking of linear epitopes via the covalent attachment, making them unable to recognize. The changes in the tertiary structure of PV may lead to the destruction of the conformational epitopes, and the IgE/IgG binding capacity is related to the integrity of antigenic, which depends on the structure of protein. Our previous study also indicated that glycosylation could significantly decrease the IgE-binding capacity of β -lg-ribose conjugate (Liu, Tu, Yang, et al., 2018). However, it is worth mentioning that the decrease of IgE/IgG binding capacity in PV-Lac is not caused by glycosylation, but lactose is involved in the immune response after being transformed into lactulose.

4 | CONCLUSIONS

In this study, different reducing sugars were used to investigate the IgE/IgG-binding capacity and conformational structure changes of PV. The results revealed that the change of IgE/IgG binding capacity was closely correlated with the structural changes. The epitope masking of proteins caused by glycosylation may be the main reason for

the decrease of IgE/IgG binding capacity. Different reducing sugar treatment methods (treatment of PV-glucose, fructose, ribose, lactose, and galactose mixtures, respectively), exhibited a distinct change in the IgE/IgG binding capacity. PV with ribose and galactose treatments promoted the reduction of IgE/IgG binding capacity, the decrease in the IgE/IgG binding capacity of PV and lactose has no relationship with glycosylation, and the IgE/IgG binding capacity of PV-Glu and PV-Fru had enhanced because of the glycosylation. Therefore, the types of reducing sugar was the main influencing factors for the glycosylated modification of PV. And in this research, glycosylation with ribose and galactose might be a promising method to reduce the IgE/IgG binding capacity of PV. However, the glycosylation reaction extent and the glycosylation sites needed to be explored by mass spectrometry to elucidate the mechanism of the reduction of IgE/IgG binding capacity at the molecular level.

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CONFLICTS OF INTEREST

The authors declared that they have no conflict of interest.

AUTHOR CONTRIBUTION

Min Zhang: Data curation; Methodology; Validation; Writing-original draft; Writing-review & editing. **Zong-cai Tu:** Funding acquisition; Project administration; Resources. **Jun Liu:** Conceptualization; Investigation; Supervision. **Yue-ming Hu:** Software. **Hui Wang:** Formal analysis; Visualization.

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