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Effect of Maillard reaction on the structural and immunological properties of recombinant silver carp parvalbumin

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ABSTRACT

The purpose of this study was to investigate the influence of Maillard reaction on the structural and immunological properties of parvalbumin (PV), the major allergen in fish. Recombinant silver carp PV (rPV) was employed and incubated with glucose at 60 °C for 72 h. The IgG/IgE binding properties of rPV were weakened after Maillard reaction as demonstrated by dot blotting. Allergenicity decrease of rPV by Maillard reaction was further confirmed on sensitized RBL-2H3 cell with the decreasing release of β-hexosaminidase, histamine and suppressing the production of interleukin-4 (IL-4) and tumor necrosis factor-α (TNF-α). Comparison of the glycation sites with documented epitopes suggested the direct blocking of conjugated carbohydrates (K_{88}, K_{97}, and K_{108}) on IgE-binding epitopes. Glycated rPV (G-rPV) exhibited slightly more resistance against pepsin digestion *in vitro*, which probably due to the formation of aggregation and the increase of hydrophobicity of G-rPV.

1. Introduction

Globally, the consumption of fish and its derivatives continues to rise due to its nutritional value and international trading, especially in China. However, fish is one of the eight prominent allergic foods that cause a series of hypersensitive reactions. In recent years, reports of adverse reactions to fish have increased, with an estimated prevalence of 0.2% in the general population (Mourad & Bahna, 2015). In Asia, fish allergy was more prevalent in Philippines (2.29%), compared to Singapore (0.26%) and Thailand (0.29%) (Connett et al., 2012). In China, occurrence of fish allergy was 0.32% in Hong Kong (Lee, Thalayasingam, & Lee, 2013), 1.32% in Taiwan (Wu et al., 2012) and 0.6% in preschool children in Guangdong province (Zeng et al., 2015).

It was reported that 92.5% of fish allergic individuals have serum-specific IgE against parvalbumins (PVs) (Sharp et al., 2015). PV is a highly stable, acidic (pI = 4.0–5.2) protein with molecular weight of 11–14 kDa, and exists in relative high concentration in fish white muscle (Kuehn, Swoboda, Arumugam, Hilger, & Hentges, 2014). Based on the amino acid sequences, two distinct phylogenetic lineages were identified and named as α- and β-PV, respectively (Goldmann & Pechere, 1977). Despite the amino acid sequence varieties, PVs from different kinds of fish share high immunological cross reactivity and similar structure (Sharp et al., 2015). It belongs to the calcium-binding EF-hand protein family with a conserved structure, in which two of the three typical helix-loop-helix domains are capable of binding Ca^{2+}. Impairment of tertiary and even secondary structure of PVs through food processing including high pressure and high temperature treatment could affect their immunological properties (Somkuti, Bublin, Breiteneder, & Smeller, 2012).

The Maillard reaction (also referred as 'glycation'), a complex series of non-enzymatic reaction between the ε-amino group of amino acid residues and the carbonyl group in reducing saccharides, is one of the most frequent reactions used in food industry. Recent studies highlighted the importance of this reaction not only in areas of food chemistry and flavor chemistry, but also in food allergy (Mueller et al., 2013). However, both the decrease (Bielikowicz et al., 2012; Seo, Karboune, L'Hocine, & Yaylayan, 2013)

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and enhancement (De Jongh et al., 2013; Heilmann et al., 2014) effects on the immunogenicity were reported on different food protein allergens. It has been found that glycation of hazelnut allergen Cor a 11 might decrease the IgE/IgG binding properties by aggregation formation of the allergen (Iwan et al., 2011). Galactan conjugation to patatin also resulted in a decrease in immunoreactivity by restricting IgE access to the epitopes, while an increase in patatin's immunoreactivity was also revealed when glycated with galactose and galactooligosaccharides (Seo, Hocine, & Karboune, 2014). These results indicated the effect of Maillard reaction on the immunoreactivity is specific for different allergens and dependent on the conjugated carbohydrates and incubating conditions.

The aim of this work was to investigate the effect of Maillard reaction on the immunoreactivity and digestibility of recombinant silver carp parvalbumin (rPV). The glycation sites and the structural modification of rPV were further determined to illustrate the possible mechanism of these changes.

2. Materials and methods

2.1. Material

2.1.1. Chemicals

D-glucose, *O*-Phthaldialdehyde (OPA), porcine pepsin and alum adjuvant, horseradish peroxidase (HRP)-conjugated goat anti-human IgE antibody were from Sigma-Aldrich (St. Louis, MO, USA). Mouse anti-silver carp PV monoclonal antibody was prepared by our laboratory as previously described (Cai et al., 2013). All other chemicals used were of analytical grade.

2.1.2. Human sera

Sera were collected from fish-allergic patients and a non-allergic individual with the approval of the local Ethics Committee of the First Affiliated Hospital of Xiamen University in Xiamen, China. Informed consent was obtained from each patient. The IgE level was measured with an ImmunoCAP assay (Phadia AB, Uppsala, Sweden), and the results were shown in Table 1. An ImmunoCAP score >0.35 kU_A/L was considered to be positive. All sera were stored at −30 °C until use.

2.2. Preparation of recombinant silver carp PV (rPV)

Silver Carp rPV was expressed and purified as previously described (Wang et al., 2014). The expression strain (BL21/pET-28a-PV), prepared previously in our laboratory, was cultured and induced with 1 mM 1-thio-β-*d*-galactopyranoside (IPTG) when the OD_{600} reached 0.6. The harvested cells were re-suspended in 20 mM Tris-HCl (pH 7.5) and sonicated. After centrifugation, the supernatant was applied to a His-Trap FF affinity column (GE Healthcare, Piscataway, USA) for purification. Target protein was analyzed by Tricine-SDS-PAGE. The protein concentrations were determined using the Bradford protein assay kit (Bio-Rad, USA), using bovine serum albumin (BSA) as the standard.

Table 1
Laboratory characterizations of three fish-allergic patients and one non-allergic individual.

Serum no.	Age	Sex	sIgE fish by ImmunoCAP (kUA/L)
A	23	F	4.50
B	19	M	1.90
C	20	M	0.71
N	21	F	0.11

2.3. Preparation of glycated PV

Maillard reaction was performed according to the previously reported method with some modifications (Teodorowicz, Fiedorowicz, Kostyra, Wichers, & Kostyra, 2013). Briefly, the samples were mixed at the ratio of rPV: glucose as 1:3 (wt/wt), freeze-dried, and incubated at 60 °C for 72 h. As a control, rPV was heated without addition of glucose. After treatment, the samples were dissolved and dialysed against 20 mM PBS (pH 7.0) at 4 °C for 24 h, and then stored at −30 °C until use.

2.4. O-Phthaldialdehyde (OPA) assay

The OPA assay was performed according to Teodorowicz et al. (2013). Protein samples were mixed with the OPA reagent in a ratio of 1:10 (v/v). The mixed solutions were incubated for 20 min at room temperature and the absorbance was measured at 340 nm. Available amino groups were estimated from a calibration curve established with L-leucine as standard.

2.5. Determination of bound sugar

The glucose contents of Maillard reaction products were determined using the anthrone sulfuric acid method (Laurentin & Edwards, 2003). The protein solutions were mixed with anthrone reagent (160 mg of anthrone dissolved in 10 mL of 14 M H_2SO_4) in the ratio of 1:10 (v/v) and the mixtures were incubated at 100 °C for 10 min. Then, the samples were cooled to 25 °C and the absorbance was measured at 620 nm. A glucose standard curve was used to calculate the sugar content.

2.6. Fluorescence and browning index analysis

Fluorescence of samples were measured at λex = 347 nm, λem = 415 nm using a spectrofluorophotometer (FP-6200, Jasco, Japan).

The extent of the brown polymer formation during the advanced and final stages of Maillard reaction was estimated spectrophotometrically by measuring the browning index at 420 nm.

2.7. Tricine-SDS-PAGE

Tricine-SDS-PAGE was carried out according to the method of Schägger (2006). After electrophoresis, gels were stained with Coomassie Blue G-250 dye, and analyzed by Image Lab Software 3.0 (Bio-Rad, USA).

2.8. Scanning electron microscopy (SEM)

Aggregation pattern of rPV after Maillard reaction was observed by SEM. Briefly, samples were directly placed on a carbon adhesive pad that was stuck to a stub. The stub was then placed in a temperature controlled sample holder. Imaging was performed on a Phenom pro scanning electron microscope (Phenom-World BV, The Netherlands) operated at 15 kV.

2.9. Dot blotting

Samples were blotted to a nitrocellulose membrane, blocked with 5% skimmed milk, and then incubated with sera (diluted at 1:4) at 4 °C overnight. HRP-labeled goat anti-human IgE antibody was then allowed to react with the nitrocellulose membrane. Antibody binding was detected by enhanced chemiluminescence (ECL).