



Protein carbonylation during electron beam irradiation may be responsible for changes in IgE binding to turbot parvalbumin

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ARTICLE INFO

Article history:

Received 12 January 2014

Accepted 28 March 2014

Available online 5 April 2014

Keywords:

EB irradiation

Parvalbumin

Allergenicity

ROS

Protein carbonyls

ABSTRACT

The present study aimed to investigate the relationship between protein carbonylation and changes of the IgE reactivity of turbot parvalbumin (PV) following electron beam (EB) irradiation. The concentration of protein carbonyls, specific IgE binding, and IgE binding inhibition between irradiated and oxidized PV were assessed. Irradiation resulted in a 3-fold enhancement in the protein carbonyl content. In purified PV irradiated with a 10-kGy dose, specific IgE binding was reduced by $91.2 \pm 6.2\%$. When raw PV was treated with reactive oxygen species (ROS), the protein carbonyl content increased 17.6-fold, with the specific IgE binding being reduced by $87.9 \pm 6.5\%$ at an ROS concentration of 10 nmol/mL. The IgE binding inhibition between irradiated and oxidized PV was investigated using an inhibition ELISA. Results showed that oxidized PV can inhibit the binding between irradiated PV and specific IgE with an IC_{50} of 8.2–58 ng according to different doses of irradiation. These findings suggest that EB irradiation reduces specific IgE binding, probably by the induction of protein carbonylation.

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1. Introduction

Seafood, especially fish, contains high quantities of protein and polyunsaturated fatty acids. Due to its nutritional value, in part due to its nutritional value, seafood is a popular and very commonly consumed food (Jin, 2008). However, recent epidemiological data suggest that fish allergies are increasing. In 2012, using a written questionnaire, Connett et al. (2012) investigated fish allergies in 14–16-year old teenagers in the Philippines. According to this report, the prevalence of fish allergies was 2.29%, which was higher than that of self-reported fish allergies in the USA (0.7%). This hypersensitivity rate was higher than that of eggs, peanuts, wheat, and tree nuts (Vierk et al., 2007). Typical fish allergy patients have clinical symptoms of diarrhea, vomiting, and even life-threatening anaphylactic shock after hypersensitivity (Priftis et al., 2008).

In order to reduce the risk of fish allergy, one efficient way is to reduce the allergenicity of fish parvalbumin (PV), which was the first identified major allergen of fish (Elsayed and Aas, 1971; Lee et al., 2007). Industrial processes such as heat (Arif and Hasnain, 2010), irradiation (Zhenxing et al., 2007), enzyme hydrolysis (Untersmayr et al., 2005), and high pressure (Liu and Xue, 2010) have been investigated to find an effective way to reduce allergen-

icity. Among these methods, irradiation is the most attractive because it is convenient, has a low cost, and is efficient. The allergenicity of certain food allergens may be reduced by irradiation, which has been confirmed in animal models (Seo et al., 2007). However, most of this work focused on gamma ray (GR) irradiation, with few researchers having used electronic beam (EB) irradiation for allergenicity reduction. Nevertheless, EB irradiation has advantages over the use of gamma irradiation: it is safe, cost-effective, easy to control and is acceptable to consumers (Park et al., 2010). Studies on the changes in the IgE-binding ability to PV after EB irradiation are imperative. Furthermore, elucidation of PV changes during EB irradiation could help to construct a stable procedure for fish allergenicity reduction.

Previous research has shown that EB irradiation can generate reactive oxygen species (ROS) in irradiated organisms (Alberti et al., 2005). Exposure of protein to ROS resulted in alterations of amino acid side chains mainly due to carbonylation (Estévez et al., 2011; Utrera and Estévez, 2012). Consequently, the protein structure is modified. Some researchers have found that protein carbonylation changes water-holding capacity and gel-forming ability during food preservation (Liu et al., 2010). It has therefore been hypothesized that the changes in IgE binding ability during irradiation may be caused by allergen carbonylation by ROS. In this study, experiments were performed to evaluate the effect of protein carbonylation on IgE binding ability to turbot PV.

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2. Materials and methods

2.1. Fish

Fresh Turbot (*Scophthalmus maximus*) of approximately 1 kg body weight was purchased from TaiDong fishery market in Qingdao, China. After removal of the guts and head, washing, and filleting, back muscles were separated into 50 ± 2.0 g pieces and sealed in sterile food-grade polyethylene plastic bags at -40°C until further use.

2.2. Chemicals

HRP-conjugated goat anti-human IgE antibody, butylated hydroxyanisole (BHA), 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox), ethylene diamine tetraacetic acid (EDTA), were procured from Sigma Chemical Co. (St. Louis, MO, USA). The enhanced chemiluminescent (ECL) substrate for immunoblotting was from Pierce (Rockford, IL). Sodium dodecyl sulfate (SDS) was procured from Bio-Rad Laboratories (Hercules, CA, USA). All other chemicals and reagents were of analytical grade.

2.3. Human sera

Sera from volunteers with fish allergies were collected from The Affiliated Hospital of Medical College Qingdao University. Turbot fish PV-specific IgE was detected in 10 serum samples. Their specific IgE level for turbot PV and total IgE level were determined by AllergyScreen (MEDIWISS Analytic GmbH, Moers, Germany). Their specific IgE levels were 1.1, 0.81, 0.44, 0.62, 0.71, 1.2, 0.64, 0.52, 0.54, 0.76 kU/l, respectively. And the total IgE levels were 186.2, 84.3, 91.5, 113.6, 98.7, 123.6, 149.7, 76.5, 94.3, 113.4 kU/l, respectively. One serum sample from a non-allergic healthy volunteer was used as a negative control. The specific IgE level and total IgE level was 0.08 kU/l and 12.1 kU/l respectively, and the patients did not show any food allergy symptoms. All serum samples were stored at -80°C until further use.

2.4. Sample irradiation

Turbot muscle samples were sealed in a sterile food-grade polyethylene plastic bag, placed in an open box with ice, and irradiated with varying doses (1 kGy, 3 kGy, 5 kGy, 7 kGy, 10 kGy, 13 kGy) by electron beam irradiation at 10 MeV. The real absorbed dose was estimated by Amber Perspex (from Harwell Company, U.K.). After irradiation, the samples were stored at -40°C until further use.

2.5. Turbot parvalbumin purification

Turbot parvalbumin (PV) was purified according to the previously described method with minor modification (Cai et al., 2010). Briefly, turbot muscles (treated or raw samples) were placed in separate aseptic homogenizer cups (Kinematica, SW-2100, China), and homogenized with 5-fold of 20 mM Tris-HCl (pH 7.4) buffer. Followed by centrifugation at 6000g for 20 min, the supernatant was separated and precipitated with 70–90% ammonium sulfate overnight. Subsequently, protein precipitate was suspended in a minimal volume of 20 mM Tris-HCl (pH 7.4) and dialyzed in the identical buffer overnight. After adjusting the pH to 4.5, the dialyzed solution was centrifuged at 8000g for 20 min, the resulting precipitate was collected and dissolved in a minimal volume of 20 mM Tris-HCl (pH 7.4) and dialyzed against the same buffer overnight. A Sephacryl S-200 column was equilibrated with 20 mM Tris-HCl, 0.15 M NaCl (pH 7.4) buffer solution. The dialyzed solution was loaded onto the gel column. This was followed by fraction collection to purify PV at a 1.0 mL/min flow rate. Eluted PV fractions were lyophilized for further experiments. The presence of PV during all purification steps was monitored by SDS-PAGE and absorbance at 220 nm. All procedures were performed at 4°C .

2.6. Protein concentration determination

Based on the Bradford method, Bio-Rad protein assay kit was used to determine protein concentration. Briefly, dye reagent dilutions were mixed separately with standard BSA and PV samples, followed by incubation at room temperature for 5 min. Concentrations were calculated after OD measurement at 595 nm. Experiments were performed in triplicate.

2.7. Parvalbumin oxidation with ROS

Fish PV was oxidized as previously described by Cui et al. (2012). In brief, fish PV solution (20 mg/mL) was incubated at $20 \pm 1^\circ\text{C}$ for 10 h in hydroxyl radical generating system (HRGS), which consists of 0.1 mM FeCl_3 , 0.1 mM ascorbic acid, and four concentrations of H_2O_2 (0.1, 1.0, 5.0, and 10.0 mM) in PBS (0.01 M, pH 7.4). The oxidation reaction was stopped by adding BHA/Trolox/EDTA (1 mM each). The negative control (nonoxidized) sample was run without HRGS components in same buffer. All treated and nontreated samples were lyophilized for further experiments.

2.8. Protein carbonylation determination

The carbonyl content in PV was determined according to Xia et al. (2009), by protein hydrazones formed by DNPH and reacted carbonyls. Absorbance of DNPH-treated samples was measured by TU-1800 spectrophotometer (Beijing Purkinje General Instrument Co. Ltd., Beijing, China) at 365 nm with HCl as control. The amount of carbonyl was expressed as nmol carbonyl per mg protein using an absorption coefficient of $2.2 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ for the protein hydrazine.

2.9. Estimation of the specific IgE binding ability by ELISA

Specific IgE binding ability to modified PV was assayed by ELISA, following the procedure of Singh et al. (2006). In brief, raw or modified (irradiated or oxidized) fish PV (1 mg/100 ml per well) was fixed in a microtiter plate (Nunc, USA), overnight at 4°C with carbonate buffer. After PBS-Tween 20 (0.05%) washing, wells were filled with 1% BSA and incubated at 37°C for 2 h, to block nonspecific sites. After washing with PBS, microtiter wells were filled with test samples (1:5 v/v diluted sera of fish-allergic patients), and incubated overnight at 4°C . Sera of healthy volunteers were used as negative controls. Horse radish peroxidase-linked anti-human IgE (1:1000 v/v) was added to microtiter wells after washing, and incubated at 37°C for 2 h. Reactions were read at 405 nm after color development by TMB and reaction termination by 2.0 M H_2SO_4 .

2.10. Effect of IgE binding inhibition between irradiated and oxidized parvalbumin

The IgE binding inhibition between irradiated and oxidized parvalbumin were evaluated by inhibition ELISA with sera from fish allergy patients. Initially, microtiter plates were coated with irradiated PV (1 $\mu\text{g}/100 \mu\text{L}$ per well) in carbonate buffer overnight at 4°C . In addition, different amounts of oxidized PV (10, 50, 100, 500, 1000 and 10,000 ng) were mixed with patient test serum (1:10 v/v) for the respective antigen, and incubated at 4°C overnight. The mixture was then added to the microtiter plate coated with irradiated PV. Control was run with normal human serum. The concentration of protein that could inhibit 50% of IgE binding was estimated using the following formula.

$$1 - \frac{\text{OD of sample with inhibitor}}{\text{OD of sample without inhibitor}} \times 100$$

2.11. Statistical analysis

Treatments were performed at least in triplicate on the same turbot muscle samples for each experiment. Data were analyzed using SPSS 19.0 software. Values are represented as mean $\pm$ SD. Analysis of variance was done to determine the significance of the changes with different treatments.

3. Results

3.1. ELISA-based comparison of specific IgE binding to PV from turbot fish samples subjected to different doses of irradiation

ELISA was performed to evaluate the IgE binding ability to fish PV using sera from patients with fish allergies. The IgE binding ability was slightly reduced by 4.7–7.1% (mean 6.0 ± 3.1 ; $p > 0.05$) when fish muscle was irradiated with a 1 kGy dose as compared with IgE binding to raw fish PV (Table 1). When the irradiation dose was increased to 5 kGy, the IgE binding ability was significantly decreased by 57.6–66.8% (mean 62.0 ± 5.6 ; $p < 0.01$ vs. 3 kGy). The maximum decline was observed when the fish was irradiated at 10 kGy: the IgE binding ability was reduced by 87.9–94.6% (mean 92.6 ± 6.1), revealing that most of the IgE binding of PV could be destroyed as compared with the raw fish muscle. The reduction in IgE binding was not linear with increased PV irradiation; when the irradiation dose increased to 13 kGy, the IgE binding of fish PV was slightly increased as compared with 10 kGy. These results show that the IgE binding ability was reduced by more than 50% when the irradiation dose was more than 5 kGy.

3.2. Changes in the protein carbonyl content following treatment with different doses of EB radiation

This study shows for the first time the development of protein carbonyls in fish PV by EB irradiation. As compared with raw PV

Table 1
Percent reduction in specific IgE binding of irradiated fish parvalbumin (PV) by ELISA.

Patients	1	2	3	4	5	6	7	8	9	Mean $\pm$ SD
1 kGy	5.4 $\pm$ 2.1	6.7 $\pm$ 2.4	7.1 $\pm$ 4.3	4.8 $\pm$ 3.1	6.1 $\pm$ 2.6	6.4 $\pm$ 2.8	5.9 $\pm$ 3.5	4.7 $\pm$ 3.6	6.7 $\pm$ 3.7	6.0 $\pm$ 3.1
3 kGy	27.3 $\pm$ 5.2	25.6 $\pm$ 4.1	32.1 $\pm$ 6.2	35.4 $\pm$ 6.3	28.4 $\pm$ 7.1	26.1 $\pm$ 2.8	23.7 $\pm$ 5.9	25.7 $\pm$ 4.7	25.4 $\pm$ 6.2	27.7 $\pm$ 5.4
5 kGy	59.4 $\pm$ 7.1	65.2 $\pm$ 5.1	63.1 $\pm$ 4.4	58.7 $\pm$ 3.8	66.4 $\pm$ 3.6	57.6 $\pm$ 5.2	58.1 $\pm$ 6.9	66.8 $\pm$ 7.3	62.4 $\pm$ 6.8	62.0 $\pm$ 5.6
7 kGy	64.1 $\pm$ 5.8	67.5 $\pm$ 6.4	69.2 $\pm$ 3.9	71.4 $\pm$ 4.1	75.6 $\pm$ 6.7	65.8 $\pm$ 5.7	74.3 $\pm$ 6.4	71.2 $\pm$ 5.2	78.6 $\pm$ 6.7	70.9 $\pm$ 5.7
10 kGy	94.2 $\pm$ 7.8	88.4 $\pm$ 6.1	89.1 $\pm$ 5.9	87.9 $\pm$ 7.2	94.6 $\pm$ 4.3	91.2 $\pm$ 4.9	87.9 $\pm$ 7.1	93.5 $\pm$ 6.7	94.1 $\pm$ 5.4	91.2 $\pm$ 6.2
13 kGy	91.3 $\pm$ 6.3	87.2 $\pm$ 7.5	81.2 $\pm$ 5.6	80.9 $\pm$ 6.4	88.4 $\pm$ 4.2	85.4 $\pm$ 5.8	88.6 $\pm$ 6.4	84.9 $\pm$ 4.8	90.1 $\pm$ 5.6	86.4 $\pm$ 5.8

Patients with significantly high specific IgE (i.e., O.D. ≥ 3) as compared to normal human sera (0.09) were selected. All data are the mean $\pm$ SD values of triplicate experiments.

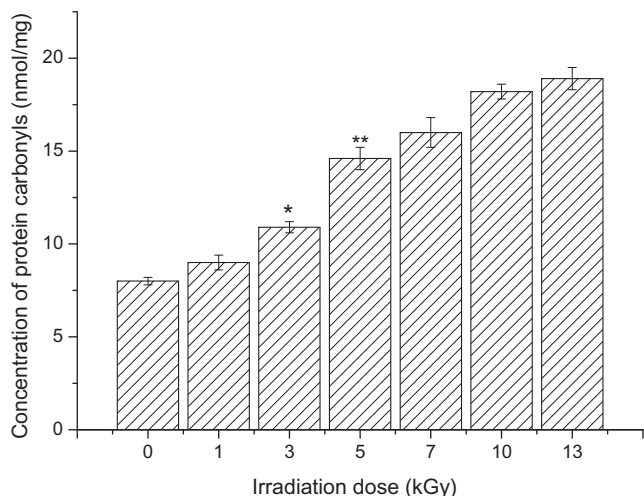


Fig. 1. Concentration of carbonyl group changes in fish PV with different irradiation doses. Results are the mean $\pm$ SD values of 3 replicated experiments. *, $p < 0.05$ vs. 1 kGy; **, $p < 0.01$ vs. 3 kGy.

(Fig. 1), the concentration of protein carbonyls in fish PV irradiated with 1 kGy was 8.0 ± 0.2 nmol/mg, which was not statistically significant ($p < 0.05$). When the irradiation dose was increased to 5 kGy, the protein carbonyl content in fish PV increased to 14.6 ± 0.6 nmol/mg, which was a significant ($p < 0.01$) change as compared with that in the 3 kGy-irradiated samples. After a 10-kGy irradiation dose, the protein carbonyl content increased almost 2-fold compared to that in raw turbot PV. At the irradiation dose of 13 kGy, the increase in carbonyls was not significantly different from that in fish subjected to the 10-kGy dose. These results indicate that protein carbonyls in PV were formed due to oxidative deamination of the side chains of arginine, proline, and/or lysine in the presence of ROS generated by EB irradiation.

3.3. Changes in specific IgE binding to parvalbumin (PV) with different concentrations of $\text{FeCl}_3/\text{H}_2\text{O}_2/\text{Asc}$

IgE binding was evaluated by ELISA using 9 individual fish allergy patients' sera. Briefly, irradiated fish PV was coated on 96-well microtiter plates. Serum from fish allergy patients was added and bound IgE antibodies were detected using biotin labeled anti-IgE antibody directed against human IgE and Streptavidin HRP. The IgE binding ability was reduced to 9.8–14.4% (mean $12.2 \pm 4.5\%$) when the concentration of free oxidative agent was 0.1 mmol/L (Table 2). The percent reduction in IgE binding ability was proportional to the concentration of ROS. When the concentration of H_2O_2 increased to 10.0 mmol/L, the IgE binding ability reduced by 84.9–89.2% (mean $87.9 \pm 6.5\%$), which indicated that only 10% of IgE binding ability remained as compared with raw fish PV. Oxidation results revealed that, when lower concentrations of H_2O_2 (up to 5.0 mmol/L) were used, the reduction of IgE binding

ability was very fast; however, the IgE binding ability was not rapidly changed with higher concentration of ROS. This may be due to the availability and sensitivity of amino acids in PV. It is in accordance with gamma irradiation in some way.

3.4. Protein carbonyl content changes with different concentrations of $\text{FeCl}_3/\text{H}_2\text{O}_2/\text{Asc}$

The concentration of protein carbonyls in PV generally increased with increasing H_2O_2 concentration (Fig. 2). Specifically, the protein carbonyl content of non-oxidized PV increased from 0.59 nmol/mg to 1.21, 3.17, 9.9, and 11.6 nmol/mg after a 10 h exposure to HRGS at 0.1, 1.0 5.0, and 10.0 nmol/mL of H_2O_2 , respectively. At 1.0 and 5.0 nmol/mL H_2O_2 , the increase in protein carbonyls was quite significant ($p < 0.01$). These data show that when the concentration of H_2O_2 increased two fold (from 5 nmol/mL to 10 nmol/mL), only a 14.7% increase in protein carbonyls was observed. These results revealed that the concentration of protein carbonyls was not directly proportional to the concentration of H_2O_2 .

3.5. Relationship between the reduction in IgE binding and the protein carbonyl content

A nearly linear relationship was seen between the reduction in IgE binding and the concentration of protein carbonyls produced by EB irradiation (Fig. 3A). Similarly, a linear relationship was observed for fish PV treated with different concentrations of HRGS (Fig. 3B). However, when the reduction in IgE binding reached 50%, the protein carbonyl concentrations were 13.8 nmol/mg and 6.11 nmol/mg in EB-irradiated and HRGS samples, respectively. These results show that protein carbonyls were also formed by ROS other than the H_2O_2 produced by EB irradiation, and the ROS affected the IgE binding ability of fish PVs weakly, if at all.

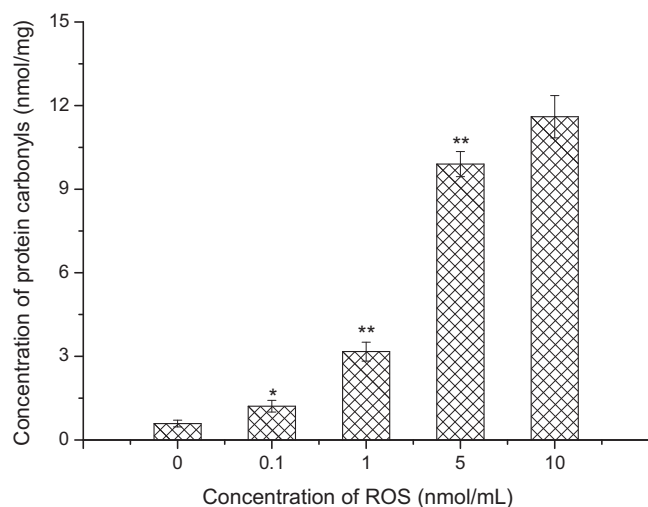
3.6. The relationship between irradiated and oxidized parvalbumin assessed by inhibition ELISA

Since the protein carbonyl content was increased by both EB irradiation and oxidation, there could be a relationship between the two treatments. The IgE binding inhibition by each treatment was assessed by inhibition ELISA. Briefly, oxidized PV was coated on a 96-well microtiter plate. Various concentrations of either oxidized or irradiated fish PV were diluted with sera from fish allergy patients and bound IgE antibodies were detected using biotin-labeled anti-IgE antibody directed against human IgE and Streptavidin HRP. The inhibition rate of oxidized PV was comparable with PV irradiated at 10 kGy and 13 kGy when IC_{50} was compared (Fig. 4). When fish PV was irradiated at 1 kGy, the inhibition rate decreased by 10-fold, with the increase in irradiation dose, the inhibition rate decreased. These results indicate that PV irradiation and oxidation could result in some shared epitopes.

Table 2

Percent reduction in specific IgE binding of oxidized turbot parvalbumin (PV) by ELISA.

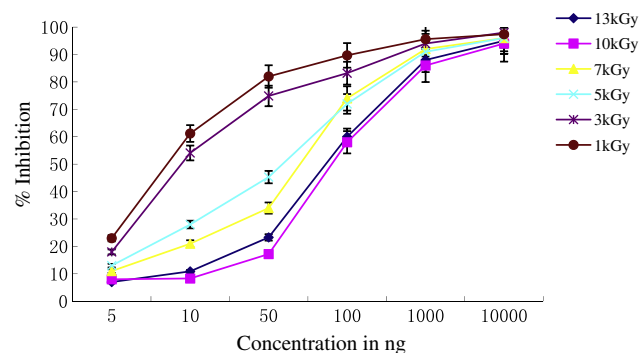
Patients	1	2	3	4	5	6	7	8	9	Mean $\pm$ SD
0.1 mmol/L	11.8 $\pm$ 4.8	12.6 $\pm$ 5.2	13.6 $\pm$ 3.6	12.8 $\pm$ 3.8	11.1 $\pm$ 4.8	14.4 $\pm$ 5.4	12.9 $\pm$ 3.5	10.7 $\pm$ 5.1	9.8 $\pm$ 4.2	12.2 $\pm$ 4.5
1.0 mmol/L	38.2 $\pm$ 6.2	35.6 $\pm$ 8.4	37.1 $\pm$ 6.7	36.2 $\pm$ 5.9	35.1 $\pm$ 3.9	36.2 $\pm$ 4.8	34.1 $\pm$ 5.9	37.2 $\pm$ 7.1	35.4 $\pm$ 6.7	36.1 $\pm$ 6.2
5.0 mmol/L	71.2 $\pm$ 4.7	75.3 $\pm$ 6.5	74.2 $\pm$ 7.2	73.2 $\pm$ 6.8	74.4 $\pm$ 5.4	77.1 $\pm$ 5.9	74.2 $\pm$ 6.4	71.8 $\pm$ 3.8	72.5 $\pm$ 8.4	73.8 $\pm$ 6.1
10.0 mmol/L	89.1 $\pm$ 5.1	88.4 $\pm$ 6.9	89.2 $\pm$ 8.1	88.6 $\pm$ 7.5	85.6 $\pm$ 6.4	87.3 $\pm$ 5.8	84.9 $\pm$ 6.3	88.6 $\pm$ 8.1	89.1 $\pm$ 4.6	87.9 $\pm$ 6.5

Patients with significantly high specific IgE (O.D.) i.e., ≥ 3 as compared to normal human sera (0.09) were selected. All data are the mean $\pm$ SD values of triplicate experiments.**Fig. 2.** Concentration of protein carbonyl group changes in parvalbumin with different HRGS. Results are the mean $\pm$ SD values of triplicate experiments. *, $p < 0.05$ vs. control; **, $p < 0.01$ vs. 0.1 or 1 nmol/mL.

4. Discussion

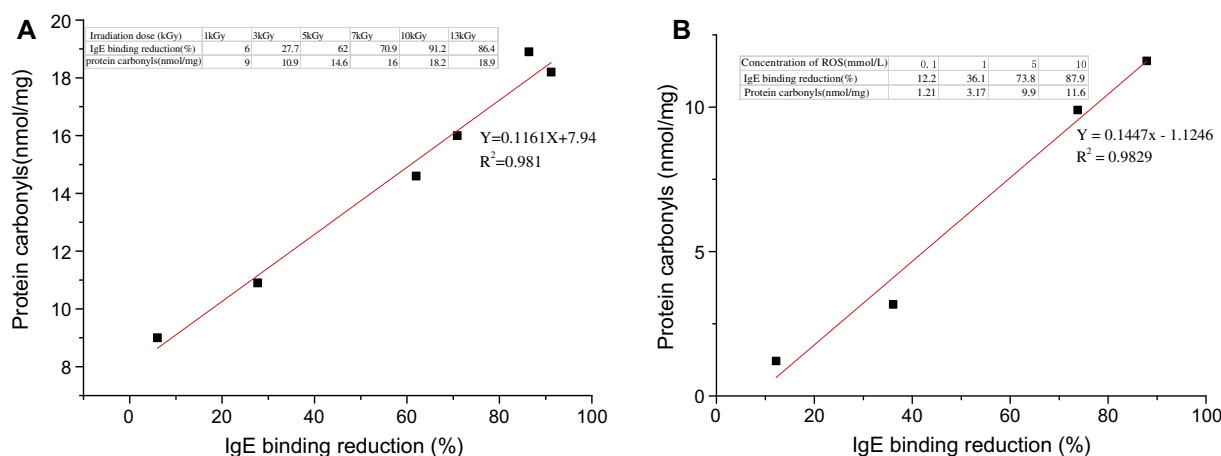
The conserved structures of food allergens, which belong to a few protein families, play a key role in determining allergenic properties. Most allergens are stable to some extent after various food processing methods involving heating, enzyme hydrolysis, pH changes, etc. (Besler et al., 2001). In order to reduce the allergenicity of food allergens, many processing methods have been studied. Among these, irradiation is most attractive due to its negligible effect on food textures (Shriver and Yang, 2011).

Until now, there have been several reports on allergenicity changes during gamma irradiation (Vaz et al., 2011; Zhenxing

**Fig. 4.** ELISA inhibition with different dose irradiated parvalbumin as inhibitor. Pooled sera from fish allergy-positive patients (diluted 1:10 v/v) were preincubated separately with 5, 10, 50, 100, 1000, or 10000 ng of their respective different dose irradiated parvalbumin as an inhibitor. ELISA was carried out on solid phase coated with oxidized parvalbumin (2 μ g/100 μ l/well) and pre-absorbed sera.

et al., 2007), however, little information was supplied on the effect of EB irradiation on allergenicity of allergens. Katial et al. (2002) have reported that EB irradiation could reduce the allergenicity of glycerinated allergen extracts, while no further research work was supplied. In the present study, the effect of irradiation on the allergenicity of fish PV and its possible mechanism were investigated.

The extent of loss of IgE binding ability depends on irradiation dose. In the present study, EB irradiation significantly reduced specific IgE binding to fish PV as compared with raw fish PV using ELISA to assay individual patient sera. With the irradiation dose increasing, the reduction in IgE binding reached $\approx 90\%$. Similar results were reported previously with gamma irradiation. Zhenxing et al. (2007) reported that gamma irradiation could reduce IgE binding to shrimp at high dose, while Vaz et al.

**Fig. 3.** (A) Relationship between the reduction in IgE binding and concentration of protein carbonyls produced by EB irradiation. (B) Relationship between the reduction in IgE binding and concentration of protein carbonyls produced by HRGS.

(2012) demonstrated that irradiation was an effective method for reducing the allergenicity of wheat-germ agglutinin. This may be attributed to ionization of molecules at random positions with the release of various radiolytic products that diffuse in the medium (mostly water with the principal radiation products H^+ and OH^-). Previous research illustrated that these ionized molecules were responsible for 99.9% of protein damage (Zbikowska et al., 2006). To some extent, these damages resulted in protein carbonylation (Kempner, 2001). Further research with the HRGS system showed that ROS (H^+ and OH^-) could increase protein carbonylation more than 10-fold while the IgE binding ability decreased by almost 90% (Rababah et al., 2004). These preliminary results demonstrated that there is a relationship between IgE binding ability and protein carbonyls of fish PV.

Protein carbonylation of fish PV is negatively correlated with its IgE binding ability. Whether PV was irradiated or treated with specific ROS, the concentration of protein carbonyls was increased along with the reduction of IgE binding ability. Previous studies reported that fish PV has several epitopes that are critical for IgE binding (Yoshida et al., 2008). Some of these epitopes contain amino acid active side chains. These active side chains can react with ROS and result in protein carbonylation. The reduction in IgE binding may be caused by epitope modification or destruction. Moreover, further analysis has confirmed that at 50% IgE binding inhibition, the concentration of protein carbonyl groups after specific ROS treatment was 0.5-fold as compared with EB irradiation. These results illustrate that protein carbonylation by ROS maybe more responsible for IgE binding changes.

Immunological inhibition between ROS treated PV and EB irradiated PV showed that protein carbonylation by ROS is due to EB irradiation. Significant inhibition was observed among the EB irradiated samples and the HRGS oxidized samples. When the irradiation dose was increased to 10 kGy, a significant increase (10-fold) in the IC_{50} was observed as compared with 1 kGy. This indicated that when fish PV was treated with EB irradiation, the reduction in IgE binding was mostly caused by H_2O_2 , which induced protein carbonylation.

Although more evidence is required to confirm that the allergen changes during irradiation are caused by protein carbonylation, this study suggests that IgE binding changes during EB irradiation might be induced by protein carbonylation. Those amino acids involved in protein carbonylation could be part of epitopes recognized by IgE. Ultimately, the epitope modification could change the IgE binding ability. In conclusion, EB irradiation could induce significant reduction in allergenic potential of turbot PV. This reduction might be due to protein carbonylation by specific ROS produced during EB irradiation. This possible mechanism will be helpful to reduce the allergenicity by EB irradiation to prepare hypoallergenic foods.

Conflict of Interest

The authors declare that there are no conflicts of interest

Transparency Document

The [Transparency document](#) associated with this article can be found in the online version.

Acknowledgment

The authors thank Dr. Tao Tian from the Allergy Centre in Qingdao for initiating a study on the prevalence of seafood allergies in China. Thanks are due to Ravindra Pawar who helped with the

linguistic revision of the manuscript. This work was supported by NSFC (31371730), Program for Changjiang Scholars and Innovative Research Team in University and National Science & Technology Pillar Program (2012BAD28B05).

References

- Alberti, A., Bertini, S., Gastaldi, G., Iannaccone, N., Macciantelli, D., Torri, G., et al., 2005. Electron beam irradiated textile cellulose fibres. ESR studies and derivatisation with glycidyl methacrylate (GMA). *Eur. Polym. J.* 41, 1787–1797.
- Arif, S.H., Hasnain, A.U., 2010. A major cross-reactive fish allergen with exceptional stability: parvalbumin. *Afr. J. Food Sci.* 4 (3), 109–114.
- Besler, Matthias, Steinhart, Hans, Paschke, Angelika, 2001. Stability of food allergens and allergenicity of processed foods. *J. Chromatogr. B: Biomed. Sci. Appl.* 756 (1), 207–228.
- Cai, Q.F., Liu, G.M., Li, T., Hara, K., Wang, X.C., Su, W.J., Cao, M.J., 2010. Purification and characterization of parvalbumins, the major allergens in red stingray (*Dasyatis akajei*). *J. Agric. Food Chem.* 58 (24), 12964–12969.
- Connett, G.J., Gerez, I., Cabrera-Morales, E.A., Yuenyongviwat, A., Ngamphaiboon, J., Chatchatee, P., Sangsupawanich, P., Soh, S.-E., Yap, G.-C., Shek, L.P.-C., Lee, B.W., 2012. A population-based study of fish allergy in the Philippines, Singapore and Thailand. *Int. Arch. Allergy Immunol.* 159 (4), 384–390.
- Cui, X., Xiong, Y.L., Kong, B., Zhao, X., Liu, N., 2012. Hydroxyl radical-stressed whey protein isolate: chemical and structural properties. *Food Bioprocess Technol.* 5 (6), 2454–2461.
- Elsayed, S., Aas, K., 1971. Isolation of purified allergens (cod) by isoelectric focusing. *Int. Arch. Allergy Immunol.* 40 (3), 428–438.
- Estévez, M., Ventanas, S., Heinonen, M., Puolanne, E., 2011. Protein carbonylation and water-holding capacity of pork subjected to frozen storage: effect of muscle type, premincing, and packaging. *J. Agric. Food Chem.* 59 (10), 5435–5443.
- Jin, S.G. (Ed.), 2008. 2002 National nutrition and health survey in Chinese residents. People's Medical Publishing House Beijing, part 10.
- Katial, R.K., Grier, T.J., Hazelhurst, D.M., Hershey, J., Engler, R.J., 2002. Deleterious effects of electron beam radiation on allergen extracts. *J. Allergy Clin. Immunol.* 110 (2), 215–219.
- Kempner, E.S., 2001. Effects of high energy electrons and gamma rays directly on protein molecules. *J. Pharm. Sci.* 90 (10), 1637–1646.
- Lee, S.J., Ju, C.C., Chu, S.L., Chien, M.S., Chan, T.H., Liao, W.L., 2007. Molecular cloning, expression and phylogenetic analyses of parvalbumin in tilapia, *Oreochromis mossambicus*. *J. Exp. Zool. Part A: Ecol. Genetics Physiol.* 307 (1), 51–61.
- Liu, R., Xue, W., 2010. High-pressure treatment with silver carp (*Hypophthalmichthys molitrix*) protein and its allergic analysis. *High Pressure Res.* 30 (3), 438–442.
- Liu, Z., Xiong, Y.L., Chen, J., 2010. Protein oxidation enhances hydration but suppresses water-holding capacity in porcine longissimus muscle. *J. Agric. Food Chem.* 58 (19), 10697–10704.
- Park, J.G., Yoon, Y., Park, J.N., Han, I.J., Song, B.S., Kim, J.H., Lee, J.W., 2010. Effects of gamma irradiation and electron beam irradiation on quality, sensory, and bacterial populations in beef sausage patties. *Meat Sci.* 85 (2), 368–372.
- Priftis, K.N., Mermiri, D., Papadopoulou, A., Papadopoulos, M., Fretzayas, A., Lagona, E., 2008. Asthma symptoms and bronchial reactivity in school children sensitized to food allergens in infancy. *J. Asthma* 45, 590–595.
- Rababah, T., Hettiarachchy, N., Horax, R., Eswaranandam, S., Mauromoustakos, A., Dickson, J., et al., 2004. Effect of electron beam irradiation and storage at 5 °C on thiobarbituric acid reactive substances and carbonyl content in chicken breast meat infused with antioxidants and selected plant extracts. *J. Agric. Food Chem.* 52, 8236–8241.
- Seo, J.H., Lee, J.W., Kim, J.H., Byun, E.B., Lee, S.Y., Kang, I.J., Byun, M.W., 2007. Reduction of allergenicity of irradiated ovalbumin in ovalbumin-allergic mice. *Radiat. Phys. Chem.* 76 (11), 1855–1857.
- Shriver, Sandra K., Yang, Wade W., 2011. Thermal and nonthermal methods for food allergen control. *Food Eng. Rev.* 3 (1), 26–43.
- Singh, A.K., Mehta, A.K., Sridhara, S., Gaur, S.N., Singh, B.P., Sarma, P.U., Arora, N., 2006. Allergenicity assessment of transgenic mustard (*Brassica juncea*) expressing bacterial cod A gene. *Allergy* 61 (4), 491–497.
- Untersmayr, E., Poulsen, L.K., Platzer, M.H., Pedersen, M.H., Boltz-Nitulescu, G., Skov, P.S., Jensen-Jarolim, E., 2005. The effects of gastric digestion on codfish allergenicity. *J. Allergy Clin. Immunol.* 115 (2), 377–382.
- Utrera, M., Estévez, M., 2012. Oxidation of myofibrillar proteins and impaired functionality: underlying mechanisms of the carbonylation pathway. *J. Agric. Food Chem.* 60 (32), 8002–8011.
- Vaz, A.F., Costa, R.M., Coelho, L.C., Oliva, M.L., Santana, L.A., Melo, A.M., Correia, M.T., 2011. Gamma irradiation as an alternative treatment to abolish allergenicity of lectins in food. *Food Chem.* 124 (4), 1289–1295.
- Vaz, A.F., Souza, M.P., Carneiro-da-Cunha, M.G., Medeiros, P.L., Melo, A.M., Aguiar, J.S., Correia, M.T., 2012. Molecular fragmentation of wheat-germ agglutinin induced by food irradiation reduces its allergenicity in sensitised mice. *Food Chem.* 132 (2), 1033–1039.
- Vierk, K.A., Koehler, K.M., Fein, S.B., Street, D.A., 2007. Prevalence of self-reported food allergy in American adults and use of food labels. *J. Allergy Clin. Immunol.* 119 (6), 1504–1510.
- Xia, X., Kong, B., Liu, Q., Liu, J., 2009. Physicochemical change and protein oxidation in porcine *Longissimus dorsi* as influenced by different freeze–thaw cycles. *Meat Sci.* 83 (2), 239–245.

- Yoshida, S., Ichimura, A., Shiomi, K., 2008. Elucidation of a major IgE epitope of Pacific mackerel parvalbumin. *Food Chem.* 111 (4), 857–861.
- Zbikowska, H.M., Nowak, P., Wachowicz, B., 2006. Protein modification caused by a high dose of gamma irradiation in cryo-sterilized plasma: protective effects of ascorbate. *Free Radic. Biol. Med.* 40 (3), 536–542.
- Zhenxing, L., Hong, L., Limin, C., Jamil, K., 2007. Impact of irradiation and thermal processing on the immunoreactivity of shrimp (*Penaeus vannamei*) proteins. *J. Sci. Food Agric.* 87 (6), 951–956.
- Zhenxing, L., Hong, L., Limin, C., Jamil, K., 2007. The influence of gamma irradiation on the allergenicity of shrimp (*Penaeus vannamei*). *J. Food Eng.* 79 (3), 945–949.