



Mechanism of the effect of 2, 2'-azobis (2-amidinopropane) dihydrochloride simulated lipid oxidation on the IgG/IgE binding ability of ovalbumin

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ABSTRACT

In this work, the mechanism of the effect of lipid oxidation on the IgG/IgE binding ability of ovalbumin (OVA) was investigated via the peroxy radicals produced by 2, 2'-azobis (2-amidinopropane) dihydrochloride to simulate lipid oxidation. Results showed that the structure of OVA unfolded partially with an increase in oxidation degree, leading to the exposure of the allergenic epitopes and increasing the IgG/IgE binding ability of OVA. Nine oxidation sites were found on the α -helix, and these sites may unwind the α -helix and expose the allergenic epitopes on the OVA surface, leading to antibody recognition and combination. Consequently, the IgG/IgE binding ability of OVA was increased. In conclusion, the allergenic capacity of OVA can be promoted by modifying peroxy radical oxidation in processing egg products.

1. Introduction

Ovalbumin (OVA), which has a molecular mass of 45 kDa, is a 385-amino acid (aa) protein that accounts for 54% of egg albumin content and is a major food allergen in hen eggs (Liao et al., 2018). Egg allergy has been the most common food allergy, surpassing milk allergy in the population of children (Mine & Yang, 2008). An allergic reaction can be classified into non-immunologic and immunologic. The latter may be further classified into immune complex complement, IgG-mediated or IgE-mediated (Li, Liu, Ye, Yang, & Tu, 2019). The IgG/IgE binding ability of proteins is typically applied to analyze anaphylaxis. The IgE linear epitopes of OVA are found nearly across the entire amino acid sequence (Elsayed and Stavseng, 1994; Mine & Yang, 2007). The most effective approach for addressing egg allergy is to completely avoid exposure to harmful compounds. However, malnutrition is an additional barrier for patients when other food allergies occur simultaneously with egg allergy. Therefore, desensitized and safe egg products should be developed for individuals with egg allergy. The following methods have been under scientific investigation in recent years to

study their effects on reducing the allergenicity of protein: heating, glycation reaction, and irradiation by obstructing certain sequences of allergenic epitopes (Liao et al., 2018). However, some methods, such as high-intensity ultrasound, can boost the potential allergenicity of proteins (Yang, Tu, Wang, Li, & Tian, 2017).

Lipids are vulnerable to oxidation and produce lipid hydroperoxide, malondialdehyde, and 4-hydroxy-2-nonenal, which react with the lysine, methionine, and histidine imidazole groups, accelerating protein oxidation. These modifications may cause protein unfolding, backbone fragmentation, cross-linking, and conformational changes, affecting the functions and properties of proteins (Duque-Estrada et al., 2019); thus, they have always been a popular topics in the food and medical science fields. Given its nutritional and processing properties (e.g., emulsifying properties), OVA, has been widely utilized in lipid-enriched food systems as an important food ingredient. OVA is susceptible to oxidative damage mediated by peroxy radicals produced by lipid oxidation during processing and storage.

The oxidation of food proteins is generally considered a negative reaction. For example, the pathogenesis of various pathological

Abbreviations: OVA, ovalbumin; AAPH, 2, 2'-azobis (2-amidinopropane) dihydrochloride; IgG, immunoglobulin G; IgE, immunoglobulin E; IgG/IgE binding ability, IgE- and IgG-binding abilities; aa, amino acid; HPLC-HCD-MS/MS, high-performance liquid chromatography-higher-energy collision dissociation-tandem mass spectrometry; ELISA, enzyme-linked immunosorbent assay; SH, sulfhydryl; UV, ultraviolet; CD, circular dichroism; OVA-N, native ovalbumin; OVA-0, heated ovalbumin; OVA-1, ovalbumin added 1 mM AAPH; OVA-5, ovalbumin added 5 mM; OVA-15, ovalbumin added 15 mM; OVA-25, ovalbumin added 25 mM; DNPH, 2, 4-dinitrophenylhydrazine; TCA, trichloroacetic acid; DTNB, 5, 5'-dithiobis- (2-nitrobenzoic acid); ANS, 1-anilinonaphthalene-8-sulfonic acid; DTT, dithiothreitol; PBS, phosphate buffer solution; PBST, PBS/Tween solution

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conditions is related to the intake of oxidized amino acids (Estévez & Luna, 2016) and oxidative stress with detrimental effects on the gel properties of OVA (Bao, Wu, Cheng, & Chi, 2017). Nevertheless, protein oxidation also has some positive effects. For example, the mild oxidation of egg white protein improves foaming ability and stability (Duan et al., 2018). Moreover, the oxidation of proline can decrease immunoreactivity by altering the structure of barley prolamin (Huang, Sontag-Strohm, Stoddard, & Kato, 2017). Although research on protein oxidation has been conducted, studies on the source, transversion, and effects of protein oxidation on food remain in their early stages in the field of chemistry (Hellwig, 2019). Therefore, research on the effects of lipid oxidation on OVA allergy is highly significant to ensure the quality and safety of food. However, existing research on this topic is limited.

2, 2'-Azobis (2-amidinopropane) dihydrochloride (AAPH) is a water-soluble azo compound that is commonly used to generate peroxy radicals under the conditions of thermal decomposition at an invariable speed. The concentration of AAPH is proportional to the amount of peroxy radicals derived by AAPH at 37 °C (Fuentes-Lemus et al., 2016). Consequently, the peroxy radicals produced by AAPH have been used as typical free radicals in lipid peroxidation.

The current study aimed to probe the mechanism of the effect of AAPH-simulated lipid oxidation on the IgG/IgE binding ability of OVA by performing conventional spectroscopy and high-performance liquid chromatography (HPLC)-higher-energy collisional dissociation-tandem (HCD) mass spectrometry (MS/MS). The IgG/IgE binding ability of OVA was estimated via indirect competitive inhibition enzyme-linked immunosorbent assay (ELISA). Oxidation degree was estimated from the contents of the carbonyl and sulfhydryl (SH) groups. The tertiary and secondary structures of the samples were analyzed on the basis of intrinsic fluorescence, ultraviolet (UV) absorption spectrum, surface hydrophobicity, and far-UV circular dichroism (CD) spectrum. Oxidation sites were determined by conducting HPLC-HCD-MS/MS at the molecular level.

2. Materials and methods

2.1. Chemicals and materials

AAPH (A101386) was obtained from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). OVA (A-5503) and pepsin (227 units/mg) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Patients' sera were bought from PlasmaLab International (Everett, WA, USA) and kept frozen (−80 °C) until analysis. Their specific IgE levels ranged from 1.6 kU/L to 24.2 kU/L (detailed information is provided in Table S1). The polyclonal antisera against OVA were obtained from six young (3 months old, approximately 2.0 kg) Japanese male rabbits. All the other chemical reagents were of analytical grade. Ultrapure water was produced using a water purification system (Millipore; Billerica, MA, USA).

2.2. Protein oxidation

The AAPH-simulated lipid oxidative modification of OVA was conducted in accordance with the method of Duan et al. (2018) with minor modifications. OVA and AAPH were dissolved in Tris – HCl buffer (pH 7.8, 10 mM). A serial concentration of AAPH was mixed with an OVA solution (40 mg/mL), and the resulting concentrations of AAPH were 0, 1, 5, 15, and 25 mM. Then, the mixed solutions were continuously shaken with sufficient oxygen in the dark at 37 °C for 24 h. The reaction processes were terminated immediately using an ice bath at 0 °C–4 °C. The OVA samples were dialyzed with deionized water at 4 °C for 36 h to dispose of the residual AAPH, freeze-dried, and stored at −20 °C. The samples were labeled as OVA-N (native OVA), OVA-0 (0 mM AAPH), OVA-1 (1 mM AAPH), OVA-5 (5 mM AAPH), OVA-15 (15 mM AAPH), and OVA-25 (25 mM AAPH).

2.3. Measurement of protein carbonyl content

Protein carbonyl content was determined using the method presented by Fu et al. (2019) with slight modifications. This method used the reaction of protein with 2, 4-dinitrophenylhydrazine (DNPH). Two aliquots of 0.2 mL of 4 mg/mL OVA samples were mixed with 0.4 mL of DNPH (10 mM, in 2 M HCl) or 0.4 mL of HCl (2 M) as a blank for 1 h at 37 °C in the dark. The reaction system was added with 0.5 mL of 20% trichloroacetic acid (TCA) and allowed to rest for 5 min to end the reaction. The mixture was centrifuged at 12000 rpm for 15 min at 4 °C to remove the supernatant from the sediment. The sediment was washed with 1 mL ethyl acetate/ethanol (1:1) and centrifuged three times as described above. The OVA samples were incubated with 1 mL of guanidine hydrochloride (6 M). The solution was centrifuged and measured at 370 nm with a UV–visible spectrophotometer. The results were expressed as nmol/mg protein and determined by the molar extinction coefficient ($22000 \text{ M}^{-1} \text{ cm}^{-1}$).

2.4. Measurement of free SH group content

The free SH group content of OVA was determined using the method of Beveridge, Toma, and Nakai (2006) with slight modifications. First, 0.4 mL samples (10 mg/mL) were dissolved in 0.6 mL Tris-glycine buffer and added with 20 µL of 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB; 10 mM). After incubation for 1 h at 37 °C in the dark, the absorbance of the OVA samples was detected at 412 nm against the blank (without DTNB and sample). The free SH group content was calculated using the following formula:

$$\text{Free SH (nmol/mg)} = \frac{A_{412}}{C} \times 73.53 \times D$$

where free SH group content was expressed in nmol/mg protein, A_{412} is the absorbance at 412 nm, C is the sample concentration in mg/mL, and D is the dilution factor.

2.5. Measurement of UV spectra

A U-2910 spectrophotometer (Hitachi; Tokyo, Japan) was used to measure the UV absorption spectrum of the samples following the method of Yang et al. (2017). The OVA sample (1 mg/mL) was measured from 240 nm to 320 nm at a speed of 800 nm/min.

2.6. Measurement of intrinsic fluorescence

Intrinsic fluorescence intensity was measured using an F-7000 fluorescence photometer (Hitachi, Tokyo, Japan) following the method of Liao et al. (2018) with minor modifications. The intrinsic fluorescence of the OVA samples (1 mg/mL) was scanned from 280 nm to 500 nm at 290 nm (excitation wavelength) with 1200 nm/min.

2.7. Determination of surface hydrophobicity (H_0)

Surface hydrophobicity was defined using 1-anilino-8-naphthalene-sulfonic acid (ANS) fluorescent probes in accordance with the method of Wang et al. (2014) with slight modifications. Each sample was diluted to four concentrations (1.0–0.125 mg/mL). Then, 8 mL of the OVA sample was mixed with 20 µL of ANS (8 mM, pH 7.4) and allowed to rest for 3 min in the dark. Fluorescence intensity was detected at 470 nm (emission wavelength) at 390 nm excitation and 5 nm slit width. The slope was used to represent the surface hydrophobicity of OVA.

2.8. Far-UV CD spectroscopy

The secondary structure of the OVA samples was determined via far-UV CD spectroscopy based on the method of Chen et al. (2018). The CD

Table 1

Protein carbonyl, free SH, and secondary structures (α -helix, β -sheet, β -turn, random coil) of ovalbumin incubated with increasing concentration of AAPH for 24 h at 37 °C.

AAPH (mM)	Protein carbonyl	Free SH	Secondary structures (%)			
	(nmol/mg)	(nmol/mg)	α -helix	β -sheet	β -turn	Random coil
OVA-N	1.36 $\pm$ 0.12e	3.75 $\pm$ 0.32d	20.60 $\pm$ 0.57a	25.51 $\pm$ 0.93a	22.90 $\pm$ 0.56a	30.98 $\pm$ 0.39b
OVA-0	1.38 $\pm$ 0.08e	4.08 $\pm$ 0.28c	20.18 $\pm$ 0.28a	25.70 $\pm$ 0.72a	22.94 $\pm$ 0.88a	30.59 $\pm$ 0.53b
OVA-1	3.45 $\pm$ 0.15d	4.13 $\pm$ 0.25b	14.93 $\pm$ 0.39b	27.91 $\pm$ 0.35b	23.42 $\pm$ 0.63a	31.45 $\pm$ 0.92b
OVA-5	3.81 $\pm$ 0.13c	4.17 $\pm$ 0.40a	14.02 $\pm$ 1.02b	33.82 $\pm$ 0.69c	23.10 $\pm$ 1.13a	28.06 $\pm$ 0.63a
OVA-15	6.18 $\pm$ 0.09b	2.69 $\pm$ 0.33e	15.40 $\pm$ 0.59b	35.81 $\pm$ 1.20d	20.89 $\pm$ 0.53b	27.26 $\pm$ 1.08a
OVA-25	11.81 $\pm$ 0.18a	2.38 $\pm$ 0.29f	11.79 $\pm$ 0.75c	37.13 $\pm$ 0.63e	19.51 $\pm$ 0.29c	31.12 $\pm$ 0.67b

All values are the mean of triplicate determinations. Values in the parenthesis are standard errors. The same letter in column indicates no difference at $p \geq 0.05$.

spectra of the OVA samples (0.1 mg/mL) was gauged using an MOS-450 spectropolarimeter (French Bio-Logic SAS; Claix, France) within a wavelength scope of 19–250 nm, a scan rate of 100 nm/min, and a path length of 1 mm. The result was reported as the mean residue ellipticity $[\theta]$ deg cm² d/mol, and the secondary structure was analyzed using Dichro Web. (<http://dichroweb.cryst.bbk.ac.uk/html/home.shtml>)

2.9. HPLC–HCD–MS/MS analysis

The oxidation sites in the OVA samples were determined via HPLC–HCD–MS/MS in accordance with the method of Yang, Liu, and Wang (2019) with minor modifications. The representative OVA sample with 25 mM AAPH heated under hydrothermal condition (OVA-25) was selected to analyze oxidation sites. First, 20 μ g of the OVA samples was dissolved in 20 μ L of urea and added with 1 μ L of dithiothreitol (DTT, 0.1 M). The samples were preserved for 5 min at 100 °C and cooled to room temperature. Subsequently, 2 μ L of the sample solution (1 μ g/ μ L) was added to 0.1 mL of iodoacetamide (0.05 M) and kept in the dark for 30 min. The OVA sample was hydrolyzed with 40 μ L of pepsin solution (1 M) in Tris-HCl buffer solution (pH 2.2). Then, the mixture solution was incubated at 4 °C for 17 h. The samples were separated via HPLC (Ultimate 3000RSLCnano). The buffer solution consisted of solvent A (0.1% formic acid in ultrapure water) and solvent B (0.1% formic acid in acetonitrile). The sample solutions were injected into an RP-C18 capillary column (Acclaim PepMap100, 100 μ m $\times$ 2 cm, Thermo Fisher Scientific) and passed through another RP-C18 analytical column (Acclaim Pep Map RSLC, 75 μ m $\times$ 15 cm, Thermo Fisher Scientific) for separation at a current speed of 300 nL/min. The peptides were eluted with 4% solvent B for 6 min and cleaned with a rational gradient of 4%–85% solvent B for 66 min. Thereafter, solvent B was decreased to 4% and maintained for 7 min. The scan range of the parent ion was from 110 m/z to 1800 m/z, and the resolution was 60,000 at m/z 200. Fragment ions were captured after positive ions fragmented via HCD.

2.10. Indirect competitive ELISA

The IgG/IgE binding ability of the native OVA sample and the samples that underwent oxidation treatment was analyzed via indirect competitive ELISA, as described previously with a few modifications (Liu et al., 2018). A 96-well microplate was coated with native OVA (2 μ g/mL, 100 μ L per well) in carbonate buffer solution and incubated for 2 h at 37 °C. The native OVA solution was discarded, and the plate was washed three times with PBST (i.e., 0.05% Tween 20 in 50 mM phosphate-buffered saline at pH 7.4). Second, the plate was blocked using 250 μ L per well of 1% fish gelatin or 2% bovine serum albumin (IgE) at 37 °C for 1 h and washed as described above. Then, 50 μ L of rabbit antisera (1:300000 in PBST) or antisera of patients (1:50 in PBST) and 50 μ L of native OVA (0.5, 1, 2, 4, 8, 16, and 32 μ g/mL) or OVA with oxidative modification were added to the microplate and incubated for 1 h at 37 °C and washed as previously described. The plate was incubated at 37 °C for 1 h with 100 μ L of goat anti-rabbit IgG-

HRB conjugate (1:5000 in PBST) or goat anti-human IgE-HRP conjugate (1:1000 in PBST). After washing, freshly prepared TMB solution (100 μ L) was added and incubated for 15 min at 37 °C. Lastly, the procedure was terminated immediately by using a sulfuric acid solution (50 μ L, 2 M). A microplate reader (Synergy H1, BioTek Instruments, Inc. U S A) was used to estimate absorbance at 450 nm. The IgG binding ability of oxidative modification OVA was computed in accordance with the standard curve of native OVA with a linear logarithmic correlation range of 0.5–32 μ g/mL. The IgE binding ability of oxidative modification OVA was expressed as the inhibition calculated using the following equation:

$$\text{Inhibition (\%)} = \left(1 - \frac{B}{B_0}\right) \times 100\%$$

where B is the absorbance value of the well with the inhibitor, and B_0 is the absorbance value of the blank control.

2.11. Statistical analysis

All of the experiments were performed in triplicate. The data were analyzed using Origin 9.0 (OriginLab Corporation; Northampton, MA, USA). SPSS 11.5 (SPSS Inc., Chicago, IL, USA) was used for one-way ANOVA and the least significant difference test, and statistical significance was set at a critical p value of < 0.05 .

3. Results and discussion

3.1. Analysis of oxidation degree

The generation of protein carbonyl is an indicator of the oxidative modification of protein by the peroxyl radicals produced by AAPH. Therefore, the extent of the oxidative modification or injury of OVA can be determined (Luceri et al., 2018). As shown in Table 1, the carbonyl content of OVA-0 was nearly the same as that of OVA-N. Meanwhile, the carbonyl level significantly increased with an increase in AAPH concentration compared with that of OVA-N ($p < 0.05$). The carbonyl content of OVA-N was 1.36 nmol/mg, and it increased to 11.82 nmol/mg in OVA-25. Peroxyl radicals produced by AAPH can induce the protein backbone of the amino acid side chains to form the peroxyl radicals of proteins. Subsequently, carbonyl derivatives were produced via amidation reaction and the reaction between amino acids (e.g., proline, lysine, and tryptophan) and secondary lipid oxidation products (Zhou, Zhao, Zhao, Sun, & Cui, 2014). The findings suggested that OVA oxidation degree was aggravated gradually with increased AAPH concentration.

The free SH content can reflect the oxidation degree of cysteine residues, which are sensitive to oxidation; by contrast, the carbonyl content cannot (Ye, Liao, Zhao, & Sun, 2013). Six cysteines were present in OVA, and Cys₇₃ and Cys₁₂₀ were composed of a disulfide bond. Previous studies have found that disulfide bonds and SH can interconvert in vitro (Thomas, Poland, & Honzatko, 1995). As shown in

Table 1, the free SH content of OVA-N was 3.75 nmol/mg, and those of OVA-0, OVA-1, and OVA-5 increased to 4.08, 4.13, and 4.17 nmol/mg, respectively. Nevertheless, the free SH contents of OVA-15 and OVA-25 decreased significantly to 2.69 nmol/mg and 2.38 nmol/mg, respectively ($p < 0.05$). Free SH contents exhibited an increasing tendency because the extent of S-S disruption was greater than that of free SH oxidation forming new S-S or the inner SH groups of OVA were exposed to the surface in the presence of 0–5 mM AAPH at 37 °C for 24 h (Duan et al., 2018); thus, OVA molecules partially unfolded. The decreasing SH content may be attributed to free SH forming disulfide bonds via oxidation. This finding agrees with the result of Headlam and Davies (2004). This phenomenon also indicated that the degree of OVA oxidation by peroxy radicals increased with an increase in AAPH concentration.

3.2. Analysis of tertiary structure

Certain aromatic amino acid residues (tryptophan, tyrosine, and phenylalanine) were present in OVA molecules, and they exhibited a maximum absorption peak in the UV region near the wavelength of 280 nm (Tu, Zhong, & Wang, 2017). Tertiary structure changes can be inferred by the changes in the UV absorption spectra of the OVA samples that underwent oxidative modification (Yamamoto et al., 2010). Fig. 1A shows that the OVA samples with oxidative modification exhibited an evident increase in the maximum UV absorbance ($\lambda_{\max}$) at 278 nm compared with that of OVA-N ($p < 0.05$). A slight blue shift of the maximum UV absorbance from 278 nm (OVA-N) to 276 nm (OVA-15, OVA-25) was observed. In general, a blue shift results from the exposure of aromatic residues (mostly tryptophan) to the protein surface and the polarity increase of the microenvironments around tryptophan (Saha, Kamilya, Bhattacharya, & Bhunia, 2014). The tertiary structure was altered by the reaction of peroxy radicals with amino

acids residues. The results suggested that the structures of the oxidative and heated OVA samples were unfolded partially, and the aromatic amino acid residues were presented on the surface of the OVA molecule via oxidative modification.

Intrinsic fluorescence is generally produced by tryptophan and tyrosine residues, and it can represent the change in a protein's tertiary structure and reflect the oxidation level of tryptophan residues (Zhao, Zhao, Chen, & Xiong, 2019). The intrinsic fluorescence intensities of the OVA samples are shown in Fig. 1B. The maximum fluorescence emission value ($\lambda_{\max}$) of OVA-N was at 348 nm. Compared with that of OVA-N, the $\lambda_{\max}$ of OVA-0 slightly decreased; however, a visible reduction was observed in the samples with added AAPH at different concentrations. Yang and Xiong (2018) reported that tryptophan residues exposed to a polar (aqueous) environment exhibited a decrease in tryptophan fluorescence intensity because the protein molecules unfolded. Among all the amino acid residues, tryptophan had the lowest one-electron potential; thus, lipid peroxidation easily attacked tryptophan residues into tryptophan peroxy radicals, accelerating protein oxidation (Zhao et al., 2019). The fluorescence quenching result indicated that OVA tertiary structures exhibited a degree of unfolding, and the degree of tryptophan residue oxidation increased with increasing AAPH concentration.

The hydrophobic interaction of hydrophobic amino acids (tryptophan, phenylalanine, and valine) in the protein molecules plays an important role in maintaining the tertiary structure of proteins; therefore, H_o is a key parameter for assessing changes in protein conformation. In the present study, the H_o of the OVA samples was explored using the fluorescence probe ANS (Ma, Gao, & Chen, 2013); the intensity of ANS was enhanced in protein hydrophobicity regions (Saricay, Wierenga, & de Vries, 2014). The changes in H_o of the OVA samples are shown in Fig. 1C. Compared with that of OVA-N, the H_o of OVA-0 markedly increased ($p < 0.05$); by contrast, the H_o of OVA-1,

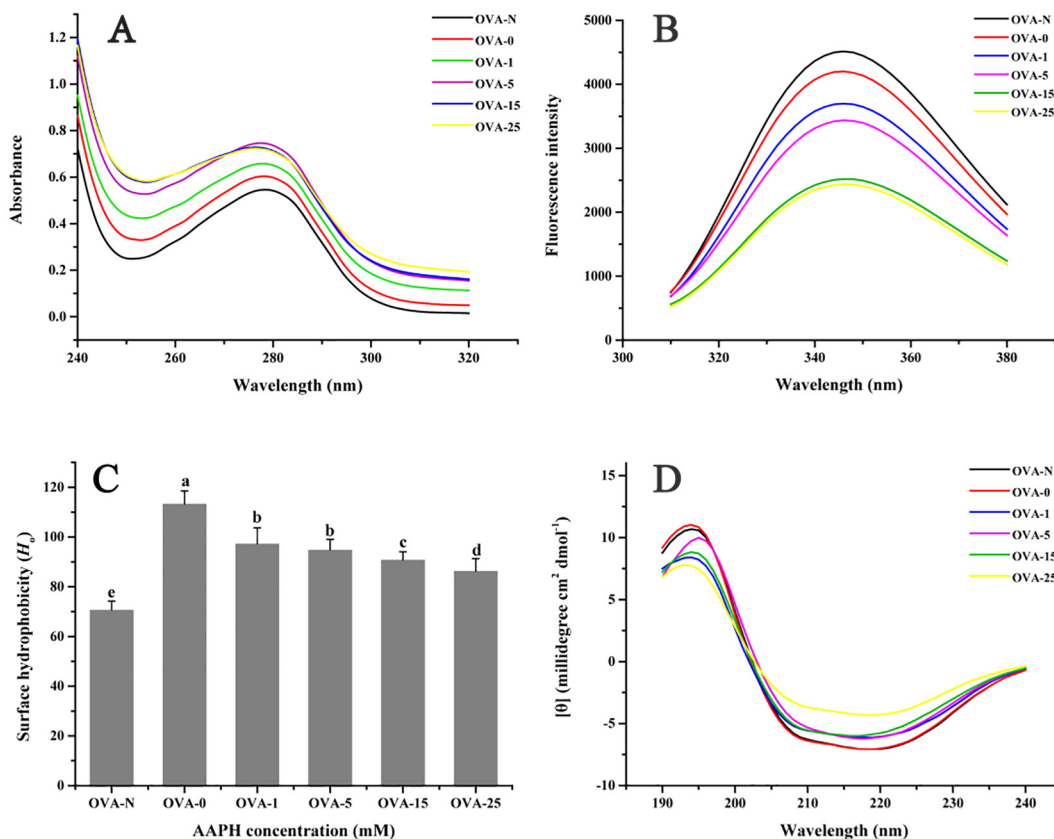


Fig. 1. Influence of incubation with different concentration AAPH at 37 °C for 24 h on the (A) UV absorption, (B) intrinsic fluorescence, (C) surface hydrophobicity (columns with different letters are significantly different ($p < 0.05$)) and (D) far-UV CD spectra of OVA.

OVA-5, OVA-15, and OVA-25 gradually decreased. Nevertheless, all of them were higher than that of OVA-N. The increase in H_0 was attributed to heating (Voutsinas, Cheung, & Nakai, 1983), and hydrophobic residues were exposed to the OVA surface. The tertiary structures of OVA were partially unfolded by peroxy radical oxidative modification, and this result agrees with those of intrinsic fluorescence and UV absorption. The decrease in H_0 shown in Fig. 1C may be caused by carbonyl compounds or other new imported hydrophilic groups; the hydrophobic groups interacted and became buried or some exposed hydrophobic residues were modified covalently (Wu, Zhang, Kong, & Hua, 2009).

3.3. Analysis of secondary structure

The CD spectra and secondary structure of the native, heated, and oxidized OVA samples are shown in Fig. 1D and Table 1. The difference between OVA-N and OVA-0 was in evident, indicating that heat under hydrothermal condition (37 °C for 24 h) exerted a negligible effect on the secondary structure of OVA. However, the α -helix content of OVA decreased from 20.60% (OVA-N) to 11.79% (OVA-25), and the β -sheet content increased from 25.51% (OVA-N) to 37.13% (OVA-25). This result demonstrated that the secondary structure of OVA changed significantly after AAPH oxidative modification at 37 °C for 24 h. Bao et al. (2017) reported similar results and found that the oxidation of OVA proteins led to a decrease in α -helix structures along with an increase in β -sheet content. Consequently, the oxidative modification of OVA may cause the partial unwinding of the α -helix structure. A possible explanation for this finding was the attack of peroxy radicals on proteins; this phenomenon decreased the number of hydrogen bonds between the NH and CO groups of amino acids that contributed to α -helix formation (Scheiner, 2005).

3.4. Analysis of IgG/IgE binding ability

In this work, indirect competitive ELISA was performed to determine the IgG/IgE binding ability of the OVA samples. The standard curve for calculating IgG binding ability is presented in Fig. S1. As shown in Fig. 2A, OVA-0 exhibited nearly the same IgG binding ability as OVA-N. However, the IgG binding abilities of OVA-1, OVA-5, OVA-15, and OVA-25 were 1.92, 2.96, 6.18, and 5.21 $\mu\text{g/mL}$, respectively, and significantly higher than those of OVA-N (1.06 $\mu\text{g/mL}$) and OVA-0 (1.07 $\mu\text{g/mL}$) ($p < 0.05$). A similar tendency was exhibited in Fig. 2B. Compared with that of OVA-N, the IgE binding abilities, expressed as the inhibition, of OVA-0, OVA-5, OVA-15, and OVA-25 nearly increased markedly ($p < 0.05$), and similar differences were found in different serum antibodies of patients. These findings indicated that heating under hydrothermal condition (37 °C for 24 h) had a negligible effect on the IgG binding capability of OVA; however, it can slightly enhance the IgE binding ability of OVA. The IgG and IgE binding abilities of OVA can be promoted significantly via peroxy radical oxidation modification. Similar results were found, suggesting that the IgG binding capability of OVA can hardly be affected under a mild condition (37 °C for 24 h) (Liao et al., 2018). IgG and IgE binding epitopes are present in nearly all of the conformations and amino acid sequences of OVA, and they can be recognized only on the protein surface. A previous work reported that the IgE antibody primarily recognized the linear epitopes of OVA and changes in the secondary and tertiary structures of proteins exerted an important effect on their IgG/IgE binding ability and were directly relevant to epitopes, including conformational and linear epitopes (Yang et al., 2017). UV absorbance, intrinsic fluorescence, and H_0 showed that the tertiary structure of the oxidative OVA samples was expanded partially via peroxy radical oxidative modification. The CD spectrum results demonstrated that the secondary structure of the OVA samples was altered by peroxy radical attack. The increase in IgG/IgE binding ability can be due to the partial exposure of some potential linear epitopes. The oxidation sites of the OVA samples were estimated via HPLC-HCD-MS/MS to further understand their relationship.

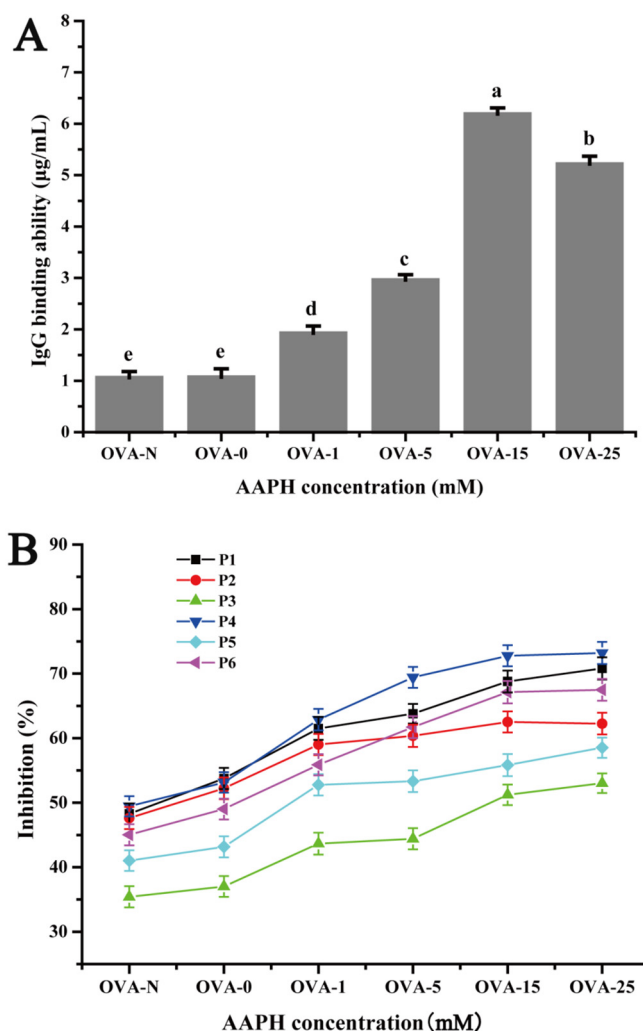


Fig. 2. Effect of incubation with different concentration AAPH at 37 °C for 24 h on IgG (A) and IgE (B) binding abilities of OVA samples (columns with different letters were significantly different ($p < 0.05$)). P1-P6 was sera of anti-egg white patients and P3 (showed the lowest inhibition) had the lowest antibody titer.

3.5. Determination of oxidation sites

Oxidation sites should be determined to further explore the mechanism of the increased IgG/IgE binding ability of peroxy radical oxidation OVA at the molecular level. OVA-25 was selected to analyze oxidation sites because of its representativeness. Pepsin was chosen to digest the OVA sample due to its highly specific ability to cleave the amide bonds of lysine and arginine. The HCD fragmentation mode was used to produce adequate b and y ions to determine MS/MS spectra in this study. The sequence coverage of the peptide map of the OVA-25 sample via HPLC-HCD-MS/MS was more than 98%, as shown in Table S2.

All the oxidation forms of protein were referred to in the report of Bachi, Dalle-Donne, and Scaloni (2013), which indicated that methionine, cysteine, lysine, arginine, tryptophan, and asparagine were easily oxidized in protein. Theoretically, if a peptide was modified with 2O, then the mass would add 32, 16, 8, and 4 Da at charge states of 1, 2, 3, and 4, respectively, for other oxidative forms. As shown in Fig. 3A, the m/z peak of the unoxidized peptide 159–172 was 738.8552²⁺, and the relative m/z peak of its oxidized peptides was 746.8535²⁺, with an m/z peak change of 7.9983 Da; thus, it has a single amino acid oxidative form. Fig. 3B shows the HCD-MS/MS spectrum of a single amino acid

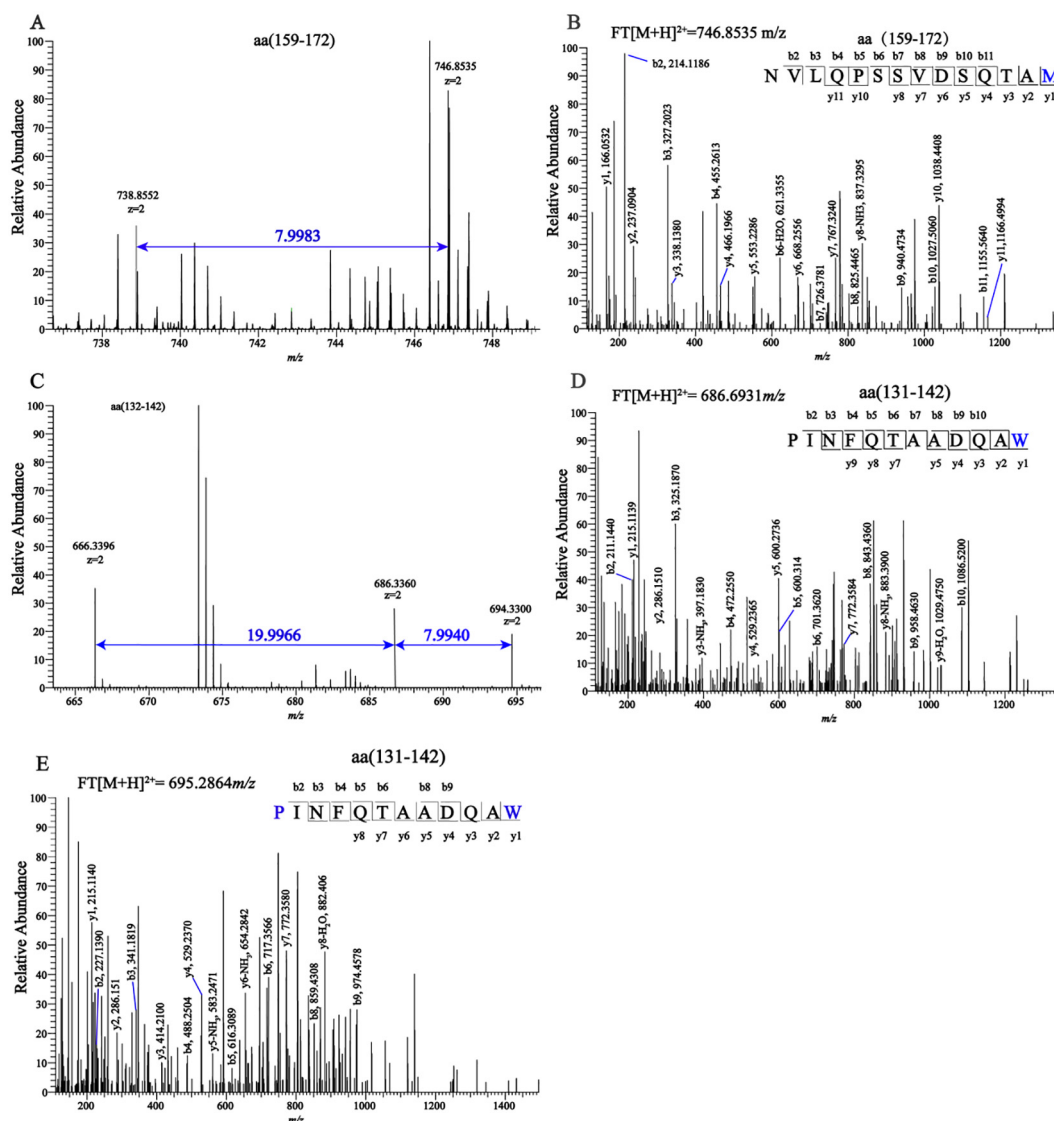


Fig. 3. Determination of oxidation sites in the peptide 159–172 and 132–142. (A) Mass spectrum of the peptide 159–172 with one oxidation site. (B) HCD-MS/MS spectrum of the peptide 159–172 at m/z 746.8535²⁺. (C) Mass spectrum of the peptide 132–142 with two oxidation sites. (D) HCD-MS/MS spectrum of the peptide 132–142 at m/z 686.6931²⁺. (E) HCD-MS/MS spectrum of the peptide 132–142 at m/z 695.2864²⁺. The sequence per peptide is depicted on the top of the spectrum and the blue amino acids are the identified oxidation sites. The b and y ions are labeled by numbers and lines. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

oxidative peptide 159–172, with a peak at m/z 738.8552²⁺, including a series of b and y ions (b2–b13, y1–y11). The oxidative site, M172, was identified through the mass difference between y1 and y2, indicating that an oxygen atom (O) combined with the methionine residues and the oxidation product was methionine sulfoxide (M + O).

Fig. 3C shows that the m/z peak of the unoxidized peptide 132–142 was 666.3396²⁺, and the homologous m/z peaks of the oxidized peptides were 686.3360²⁺ and 694.3300²⁺, with an m/z peak increase of 19.9966 Da and 7.9940 Da, respectively. This finding showed the single and doubly amino acid oxidative forms of peptides. The b and y ions of the HCD-MS/MS spectrum shown in Fig. 3D were matched well with peptide 132–142 with an m/z peak of 666.3396²⁺, and the oxidation site R142 was identified. Similarly, P131 and R142 were determined from the HCD-MS/MS spectrum of the doubly amino acid oxidative form of peptide as shown in Fig. 3E. An oxygen atom and two carbon atoms (+19.9966) were combined in the arginine residues (R142), and the oxidation product was glyoxal-derived imidazolone (R + 2C + O). P131 was modified by an oxygen atom (+7.9940), and the oxidation product was glutamic semialdehyde (P + O) or hydroxyproline

(P + O).

A total of 23 oxidative sites at C30, R50, K55, K92, T91, N88, P131, R142, N146, M172, W184, M196, K206, M211, N260, W267, M273, R276, R284, M287, K322, N335, R359, and H370 were detected. Their oxidative forms and products are provided in Table 2. The results suggested that HPLC–HCD-MS/MS can precisely identify the location of the oxidative sites of OVA. However, it can not discriminate the isomers of the products. Consequently, the tryptophan oxidation products attached to two oxygen atoms (W + 2O) can not be ascertained as *N*-formylkynurenine, dioxindolylalanine, or dihydroxytryptophan. Similarly, whether the oxidation product of proline was hydroxyproline or glutamic semialdehyde was not confirmed.

3.6. Oxidation enhanced the IgG/IgE binding abilities of OVA

In this work, the IgG/IgE binding ability of OVA was significantly boosted via peroxy radical oxidation modification. Consequently, the effect of the changes in structure induced by peroxy radical oxidation on allergy epitopes should be discussed to explore the mechanism for

Table 2
The identified oxidative peptides and products in OVA-25.

Peptide location	Sequence	m/z	Theor.	Exp.	Oxidation form localization	Product
30–33	(Y)CPIA(I)	403.2011	419.1959	419.1980	C + O	sulfenic acid
43–55	(Y)LGAKDSTRQTQINK(V)	716.3991 ²⁺	737.4046 ²⁺	737.6611 ²⁺	R50 + 2H + 2C + O	glyoxal-derived hydroimidazolone
51–58	(R)TQINKVVR(F)	479.2958 ²⁺	487.2931 ²⁺	487.6979 ²⁺	K55 + O	hydroxylysine
85–92	(R)DILNQITK(P)	472.7746 ²⁺	488.7746 ²⁺	488.5674 ²⁺	K92 + O; T91 + O	hydroxythreonine; hydroxylysine
85–99	(R)DILNQITKPNVDVYSF(S)	883.9546 ²⁺	884.4436 ²⁺	884.4546 ²⁺	N88-H-N + O	3-hydroxyasparagine
131–142	(E)PINFQTAAQAR(E)	666.3396 ²⁺	686.3362 ²⁺	686.6931 ²⁺	P131 + O; R142 + 2C + O	Hydroxyproline/glutamic/semialdehyde;glyoxal-derived imidazolone
143–158	(R)ELINSWVESQTNGHIR(N)	620.3278 ³⁺	620.9907 ³⁺	621.0593 ³⁺	N146-N + O	3-hydroxyasparagine
159–172	(R)NVLPQSSVDSQTAM(V)	738.8552 ²⁺	746.851 ²⁺	746.8535 ²⁺	M172 + O	methionine sulfoxide
182–186	(K)GLWEK(A)	632.3427	636.3352	636.6554	W184-C + O	kynurenine
187–199	(A)AFKDEDITQAMPFR(V)	519.2465 ³⁺	524.5768 ³⁺	524.5773 ³⁺	M196 + O	methionine sulfoxide
200–212	(R)VTEQESKPVQMMY(Q)	785.3696 ²⁺	793.3655 ²⁺	793.3689 ²⁺	K206 + O	hydroxylysine
211–218	(M)MYQIGLFR(V)	514.2736 ²⁺	522.2708 ²⁺	522.2709 ²⁺	M211 + O	methionine sulfoxide
255–263	(Q)LESINFEK(L)	546.8021 ²⁺	547.2924 ²⁺	547.2541 ²⁺	N260-H-N + O	3-hydroxyasparagine
264–276	(K)LTEWTSNNMEER(K)	791.3658 ²⁺	815.3567 ²⁺	815.3583 ²⁺	W267 + 2O; M273 + O	N-formylkynurenine/dioxindolylalanine, dihydroxytryptophan; methionine sulfoxide
268–276	(W)TSSNNMEER(K)	526.7383 ²⁺	547.7428 ²⁺	547.7471 ²⁺	R276 + 2H + 2C + O	glyoxal-derived hydroimidazolone
279–284	(I)KVVLPK(M)	388.2450 ²⁺	409.2502 ²⁺	409.2319 ²⁺	R284 + 2H + 2C + O	glyoxal-derived hydroimidazolone
286–290	(M)KMEEK(Y)	664.3311	696.3328	696.3233	M287 + 2O	methionine sulfone
307–322	(F)SSSANLSGSSAESLK(I)	769.3906 ²⁺	777.3863 ²⁺	777.3585 ²⁺	K322 + O	hydroxylysine
323–339	(K)ISQAVHAAHAEINEAGR(E)	887.4550 ²⁺	887.9452 ²⁺	887.9462 ²⁺	N335-H-N + O	3-hydroxyasparagine
353–359	(A)SVSEEFK(A)	427.2072 ²⁺	448.2114 ²⁺	448.2115 ²⁺	R359 + 2H + 2 + O	glyoxal-derived hydroimidazolone
365–372	(F)LFCKIHIA(T)	472.7746 ²⁺	480.7704 ²⁺	480.7717 ²⁺	H370 + O	2-oxohistidine

this phenomenon. The methods used for analysis were conventional spectrometry and HPLC–HCD–MS/MS at the molecular level.

The results indicated that the increasing IgG/IgE binding ability of OVA was closely related to variations in its structure. As shown in Fig. 4A, the oxidation of the W184 and W267 of hydrophobic amino acid will yield kynurenine and dioxindolylalanine. Oxidation products that introduce hydrophilic groups can be used to explain the decrease in surface hydrophobicity (H_o) and tryptophan intrinsic fluorescence quenching of OVA-25 compared with OVA-0. However, the H_o of the oxidation modification samples was higher than that of OVA-N; thus, the increasing IgG/IgE binding ability of OVA will be affected by increasing H_o . Many hydrophobic amino acids are present in the domains of antigen-antibody binding; they play an important role in antigen-antibody binding and cross-reactions through hydrophobic interactions (Zhang, Ma, Gao, & Dai, 2010). The oxidation of some charged

amino acids, such as R142, R284, R276, and R359, may destroy the electrostatic interactions of molecules and dissociate noncovalent bonds (Wang et al., 2018). Thus, the tertiary structure of OVA can become loose. Many linear epitopes of IgG and IgE can be exposed on the surface of OVA, strengthening the identified allergic epitopes that combined with IgG/IgE. Moreover, the oxidation sites of R50, K55, N88, T91, K92, R142, N146, N260, and W267 were found in the α -helix of OVA with oxidation modification. This phenomenon may be the reason for the uncoiling of the α -helix. The amino acids of R50 and W267 attacked by peroxy radicals can develop into glyoxal-derived hydroimidazolone and N-formylkynurenine. They can enlarge R groups of arginine and tryptophan, resulting in steric hindrance and blocking hydrogen bond formation between the NH and CO groups of amino acids. The oxidation of N88 and T91 can uncoil the α -helix of aa (81–91) and exposed the allergic peptide of aa (77–84) from the α -helix

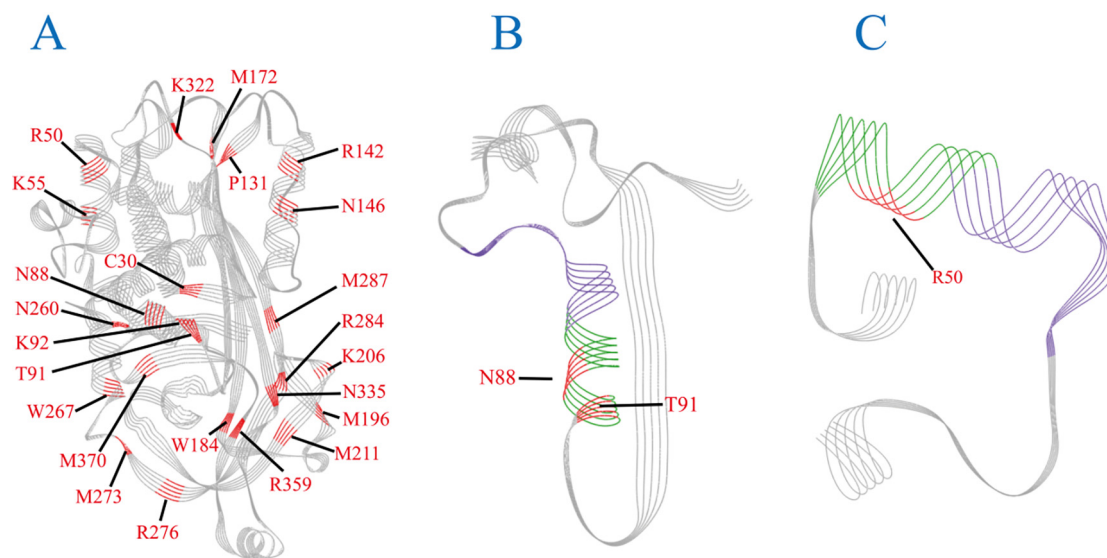


Fig. 4. (A) Ribbon diagram [Protein Data Bank (PDB) code 1JTI] of OVA. (B) The partial ribbon diagram of OVA. The colored α -helix is peptide 81–91. Purple is the allergic peptide of peptide 77–84. (C) The partial ribbon diagram of OVA. The colored α -helix is peptide 47–57. Purple is the allergic peptide of peptide 53–60. Gray, framework of OVA; red, oxidation sites of OVA-25. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in Fig. 4B. As shown in Fig. 4C, the oxidation of R50 will cause the α -helix of aa (47–57) to uncoil and the allergenic peptide of aa (53–60) will be exposed. In general, IgG and IgE could not easily identify the epitopes in the α -helix (Matsuo, Yokooji, & Taogoshi, 2015); thus, the uncoiling of the α -helix structure may contribute to the recognition and combination of epitopes in the α -helix by antibodies. Previous research has shown that water molecules in the antigen–antibody and light and heavy chain variable domain interfaces can supplement the imperfect complementarity of the interfaces by forming a hydrogen bond network between the antigen and the white lysozyme antibody of hen egg with these water molecules (Kondo, Shiroishi, Matsushima, Tsumoto, & Kumagai, 1999). Therefore, we proposed an interesting hypothesis that the oxidation of K322 and N335 may not have destroyed the linear epitope of ³²³ISQAVHAAHA³³², however, it may promote hydrogen bond formation between water molecules and the IgE antibody or the carbonyl compounds yielded by K 322 and N 335. This phenomenon can be helpful in increasing the combination of allergic epitopes with IgE.

4. Conclusion

This work was the first to discuss the mechanism of lipid oxidation simulated by peroxy radical effect on the IgG/IgE binding ability of OVA. This mechanism was explored through the oxidation degree and changes in the secondary and tertiary structures and oxidation sites. The results indicated that peroxy radical oxidation can expose OVA to allergenic epitopes by inducing changes in its tertiary and secondary structures; consequently, the chances of IgG/IgE recognition and combination with allergenic epitopes were increased. The IgG/IgE binding ability of OVA will increase with an increase in oxidation degree. The limitations of the present study were that the serum of patients without clinical allergy to egg after egg consumption was not used. Therefore, future research should determine the IgE binding ability of OVA using the sera from patients without clinical allergy to egg after taking egg, and that the results should be further validated using sera from patients with clinical egg allergy after their consumption and with data of positive specific IgE against OVA. Moreover, molecular docking, animal and cell model experiments should be conducted in the future to further confirm the effects of peroxy radical oxidation on OVA sensitization. Research that combines peroxy radical oxidation modification with other methods to reduce sensitivity to OVA should be conducted to provide theoretical references for the development, processing, and storage of products with low sensitization effects.

CRedit authorship contribution statement

Jing-jing Zhang: Methodology, Data curation, Writing - original draft, Writing - review & editing, Validation. **Zong-cai Tu:** Funding acquisition, Project administration, Resources. **Hui Wang:** Conceptualization, Investigation, Supervision. **Yue-ming Hu:** Visualization. **Peng-cheng Du:** Software. **Yi-peng Yang:** Formal analysis.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2020.127037>.

References

- Bachi, A., Dalle-Donne, I., & Scaloni, A. (2013). Redox proteomics: Chemical principles, methodological approaches and biological/biomedical promises. *Chemical Reviews*, 113(1), 596–698.
- Bao, Z., Wu, J., Cheng, Y., & Chi, Y. (2017). Effects of lipid peroxide on the structure and gel properties of ovalbumin. *Process Biochemistry*, 57, 124–130.
- Beveridge, T., Toma, S. J., & Nakai, S. (2006). Determination of SH- and SS-groups in some food proteins using Ellman's Reagent. *Journal of Food Science*, 39(1), 49–51.
- Chen, Y., Tu, Z. C., Wang, H., Liu, G. X., Liao, Z. W., & Zhang, L. (2018). LC-Orbitrap MS analysis of the glycation modification effects of ovalbumin during freeze-drying with three reducing sugar additives. *Food Chemistry*, 268, 171–178.
- Duan, X., Li, M., Shao, J., Chen, H., Xu, X., Jin, Z., & Liu, X. (2018). Effect of oxidative modification on structural and foaming properties of egg white protein. *Food Hydrocolloids*, 75, 223–228.
- Duque-Estrada, P., Berton-Carabin, C. C., Nieuwkoop, M., Dekkers, B. L., Janssen, A. E. M., & van der Goot, A. J. (2019). Protein oxidation and in vitro gastric digestion of processed soy-based matrices. *Journal of Agricultural and Food Chemistry*, 67(34), 9591–9600.
- Elsayed S, & Stavseng L. (1994). Epitope mapping of region 11–70 of ovalbumin (Gal d I) using five synthetic peptides. *International Archives of Allergy and Immunology*, 104(1), 65–71.
- Estévez, M., & Luna, C. (2016). Dietary protein oxidation: A silent threat to human health? *Critical Reviews in Food Science and Nutrition*, 57(17), 3781.
- Fu, Q., Liu, R., Wang, H., Hua, C., Song, S., Zhou, G., & Zhang, W. (2019). Effects of oxidation in vitro on structures and functions of myofibrillar protein from beef muscles. *Journal of Agricultural Food Chemistry*, 67(20), 5866–5873.
- Fuentes-Lemus, E., Dorta, E., Escobar, E., Aspée, A., Pino, E., Abasq, M. L., ... López-Alarcón, C. (2016). Oxidation of free, peptide and protein tryptophan residues mediated by AAPH-derived free radicals: Role of alkoxyl and peroxy radicals. *RSC Advances*, 6(63), 57948–57955.
- Headlam, H. A., & Davies, M. J. (2004). Markers of protein oxidation: Different oxidants give rise to variable yields of bound and released carbonyl products. *Free Radical Biology & Medicine*, 36(9), 1175–1184.
- Hellwig, M. (2019). The chemistry of protein oxidation in food. *Angewandte Chemie International Edition*, 58(47), 16742–16763.
- Huang, X., Sontag-Strohm, T., Stoddard, F. L., & Kato, Y. (2017). Oxidation of proline decreases immunoreactivity and alters structure of barley prolamin. *Food Chemistry*, 214, 597–605.
- Kondo, H., Shiroishi, M., Matsushima, M., Tsumoto, K., & Kumagai, I. (1999). Crystal structure of anti-Hen egg white lysozyme antibody (HyHEL-10) Fv-antigen complex. *Journal of Biological Chemistry*, 274(39), 27623–27631.
- Li, J. L., Liu, J., Ye, Y. H., Yang, P., & Tu, Z. C. (2019). Reduced IgE/IgG binding capacities of bovine alpha-Lactalbumin by glycation after dynamic high-pressure micro-fluidization pretreatment evaluated by high resolution mass spectrometry. *Food Chemistry*, 299, 125–166.
- Liao, Z. W., Ye, Y. H., Wang, H., Chen, Y., Sha, X. M., Zhang, L., ... Tu, Z. C. (2018). The Mechanism of decreased IgG/IgE-Binding of ovalbumin by preheating treatment combined with glycation identified by liquid chromatography and high-resolution Mass Spectrometry. *Journal of Agricultural Food Chemistry*, 66(41), 10693–10702.
- Liu, G.-X., Tu, Z.-C., Yang, W., Wang, H., Zhang, L., Ma, D., ... Li, X. (2018). Investigation into allergenicity reduction and glycation sites of glycated β -lactoglobulin with ultrasound pretreatment by high-resolution mass spectrometry. *Food Chemistry*, 252, 99–107.
- Luceri, C., Bigagli, E., Femia, A. P., Caderni, G., Giovannelli, L., & Lodovici, M. (2018). Aging related changes in circulating reactive oxygen species (ROS) and protein carbonyls are indicative of liver oxidative injury. *Toxicology Reports*, 5, 141–145.
- Ma, X. J., Gao, J. Y., & Chen, H. B. (2013). Combined effect of glycation and sodium carbonate-bicarbonate buffer concentration on IgG binding, IgE binding and conformation of ovalbumin. *Journal of the Science of Food and Agriculture*, 93(13), 3209–3215.
- Matsuo, H., Yokooji, T., & Taogoshi, T. (2015). Common food allergens and their IgE-binding epitopes. *Allergology International*, 64(4), 332–343.
- Mine, Y., & Yang, M. (2007). Epitope characterization of ovalbumin in BALB/c mice using different entry routes. *Biochimica et Biophysica Acta*, 1774(2), 200–212.
- Mine, Y., & Yang, M. (2008). Recent advances in the understanding of egg allergens: Basic, industrial, and clinical perspectives. *Journal of Agricultural Food Chemistry*, 56(13), 4874–4900.
- Saha, S., Kamilya, T., Bhattacharya, R., & Bhunia, A. K. J. S. o. A. M. (2014). Unfolding of blood plasma albumin protein in interaction with CdS nanoparticles. *Science of Advanced Materials*, 6(1), 56–62.
- Saricay, Y., Wierenga, P. A., & de Vries, R. (2014). Changes in protein conformation and surface hydrophobicity upon peroxidase-catalyzed cross-linking of apo-alpha-lactalbumin. *Journal of Agricultural Food Chemistry*, 62(38), 9345–9352.
- Scheiner, S. (2005). Relative strengths of NH, O and CH, O Hydrogen bonds between polypeptide chain segments. *The Journal of Physical Chemistry B*, 109(33), 16132–16141.
- Thomas, J. A., Poland, B., & Honzatko, R. (1995). Protein sulfhydryls and their role in the antioxidant function of protein S-thiolation. *Archives of Biochemistry Biophysics*,

- 319(1), 1–9.
- Tu, Z. C., Zhong, B. Z., & Wang, H. (2017). Identification of glycosylated sites in ovalbumin under freeze-drying processing by liquid chromatography high-resolution mass spectrometry. *Food Chemistry*, 226, 1–7.
- Voutsinas, L. P., Cheung, E., & Nakai, S. (1983). Relationships of hydrophobicity to emulsifying properties of heat denatured proteins. *Journal of Food Science*, 48(1), 26–32.
- Wang, W., Shen, M., Liu, S., Jiang, L., Song, Q., & Xie, J. (2018). Gel properties and interactions of mesona blumes polysaccharide-soy protein isolates mixed gel: The effect of salt addition. *Scientific and Technological Aspects of Industrially Important Polysaccharides*, 192, 193–201.
- Wang, P., Tao, H., Wu, F., Yang, N., Chen, F., Jin, Z., & Xu, X. (2014). Effect of frozen storage on the foaming properties of wheat gliadin. *Food Chemistry*, 164, 44–49.
- Wu, W., Zhang, C., Kong, X., & Hua, Y. (2009). Oxidative modification of soy protein by peroxyl radicals. *Food Chemistry*, 116(1), 295–301.
- Yamamoto, S., Takanohashi, K., Hara, T., Odani, S., Suzuki, A., & Nishiumi, T. (2010). Effects of a high-pressure treatment on the wheat alpha-amylase inhibitor and its relationship to elimination of allergenicity. *Journal of Physics: Conference Series*, 215, 012170.
- Yang, Y., Liu, G., & Wang, H. (2019). Investigation of the mechanism of conformational alteration in ovalbumin as induced by glycation with different monoses through conventional spectrometry and liquid chromatography high-resolution Mass Spectrometry. *Journal of Agricultural Food Chemistry*, 67(11), 3096–3105.
- Yang, W., Tu, Z. C., Wang, H., Li, X., & Tian, M. (2017). High-intensity ultrasound enhances the immunoglobulin (Ig)G and IgE binding of ovalbumin. *Journal of the Science of Food and Agriculture*, 97(9), 2714–2720.
- Yang, W., Tu, Z., Wang, H., Zhang, L., Xu, S., Niu, C., ... Kaltashov, I. A. (2017). Mechanism of reduction in IgG and IgE binding of beta-lactoglobulin induced by ultrasound pretreatment combined with dry-state glycation: A study using conventional spectrometry and high-resolution Mass Spectrometry. *Journal of Agricultural and Food Chemistry*, 65(36), 8018–8027.
- Yang, J., & Xiong, Y. L. (2018). Comparative time-course of lipid and myofibrillar protein oxidation in different biphasic systems under hydroxyl radical stress. *Food Chemistry*, 243, 231–238.
- Ye, L., Liao, Y., Zhao, M., & Sun, W. (2013). Effect of protein oxidation on the conformational properties of peanut protein isolate. *Journal of Chemistry*, 2013, 1–6.
- Zhang, Y. F., Ma, Y., Gao, Z.-X., & Dai, S.-G. (2010). Predicting the cross-reactivities of polycyclic aromatic hydrocarbons in ELISA by regression analysis and CoMFA methods. *Analytical Bioanalytical Chemistry*, 397(6), 2551–2557.
- Zhao, X., Zhao, Q., Chen, H., & Xiong, H. (2019). Distribution and effects of natural selenium in soybean proteins and its protective role in soybean beta-conglycinin (7S globulins) under AAPH-induced oxidative stress. *Food Chemistry*, 272, 201–209.
- Zhou, F., Zhao, M., Zhao, H., Sun, W., & Cui, C. (2014). Effects of oxidative modification on gel properties of isolated porcine myofibrillar protein by peroxyl radicals. *Meat Science*, 96(4), 1432–1439.

Table S1 Information of EA patients

No.	Age	Gender	Test Type	Allergen	Other allergen	IgE (anti OVA) (kU/L)
P1	28	Male	ImmunoCAP	Egg white	Peanut, Soybean	21.3
P2	51	Female	ImmunoCAP	Egg white	Walnut, Cottonwood	11.5
P3	23	Male	ImmunoCAP	Egg white	Pistachio, Olive	1.6
P4	49	Male	ImmunoCAP	Egg white	Codfish, Wheat	24.2
P5	33	Male	ImmunoCAP	Egg white	Wheat, Shrimp	10.8
P6	16	Male	ImmunoCAP	Egg white	Peanut, Soybean	13.7

Allergy survey results showed that all the above patients had a recent history of anaphylaxis after egg consumption.

Table S2 Peptide mapping of OVA-25

No.	m/z	MH ⁺	MH ⁺	Δ ppm	Start	End	Sequence
		experimental	theoretical				
1	385.256	385.256	385.2558	0.571	282	284	(Y) <u>L</u> PR(M)
2	388.2542	388.2542	388.2554	-3.21	43	46	(Y) <u>L</u> GAK(D)
3	393.2498	393.2498	393.2496	0.427	14	16	(D) <u>V</u> FK(E)
4	393.2498	393.2498	393.2496	0.427	179	181	(I) <u>V</u> FK(G)
5	403.2011	403.2011	403.201	0.329	30	33	(Y) <u>C</u> PIA(I)
6	419.24	419.24	419.2401	-0.31	197	199	(M) <u>P</u> FR(V)
7	421.256	421.256	421.2558	0.522	57	59	(V) <u>V</u> RF(D)
8	421.256	421.256	421.2558	0.522	198	200	(P) <u>F</u> RV(T)

9	421.256	421.256	421.2558	0.522	217	219	(L) <u>FRV(A)</u>
10	462.2351	462.2351	462.2347	0.843	184	186	(L) <u>WEK(A)</u>
11	466.2298	466.2298	466.2296	0.376	134	137	(N) <u>FQTA(A)</u>
12	478.2299	478.2299	478.2296	0.575	233	237	(L) <u>PFASG(T)</u>
13	492.2931	492.2931	492.2929	0.419	215	218	(I) <u>GLFR(V)</u>
14	492.2931	492.2931	492.2929	0.419	217	220	(L) <u>FRVA(S)</u>
15	503.2981	503.2981	503.2976	0.904	181	184	(F) <u>KGLW(E)</u>
16	506.334	506.334	506.3337	0.6	178	181	(A) <u>IVFK(G)</u>
17	515.2251	515.2251	515.2249	0.439	361	364	(A) <u>DHPF(L)</u>
18	548.3203	548.3203	548.3191	2.17	110	113	(E) <u>RYPI(L)</u>
19	548.3203	548.3203	548.3191	2.17	281	284	(V) <u>YLPR(M)</u>
20	551.3195	551.3195	551.3188	1.32	42	46	(V) <u>YLGAK(D)</u>

21	561.3404	561.3404	561.3395	1.59	61	65	(D) <u>KLP</u> G(F)
22	574.3199	574.3199	574.3195	0.692	310	315	(S) <u>ANLS</u> G(I)
23	577.372	577.372	577.3708	2.06	177	181	(N) <u>AIV</u> F(K)
24	586.2626	586.2626	586.262	1.04	360	364	(R) <u>ADH</u> P(F)
25	605.3774	605.3774	605.377	0.731	214	218	(Q) <u>IGL</u> F(R)
26	605.3774	605.3774	605.377	0.731	216	220	(G) <u>LFR</u> VA(S)
27	618.3254	618.3254	618.3246	1.31	131	135	(E) <u>PIN</u> F(Q)
28	618.3254	618.3254	618.3246	1.31	145	149	(L) <u>INS</u> W(V)
29	632.3427	632.3427	632.3402	3.89	144	148	(E) <u>LINS</u> W(V)
30	632.3427	632.3427	632.3402	3.89	181	185	(F) <u>KGL</u> WE(K)
31	632.3427	632.3427	632.3402	3.89	182	186	(K) <u>GLW</u> E(K)
32	634.3408	634.3408	634.3406	0.265	312	318	(N) <u>LSGI</u> SSA(E)

33	634.3408	634.3408	634.3406	0.265	317	322	(S) <u>SAESLK(I)</u>
34	635.3773	635.3773	635.3763	1.59	14	18	(D) <u>VFKEL(K)</u>
35	650.3883	650.3883	650.3872	1.71	41	46	(M) <u>VYLGAK(D)</u>
36	657.3592	657.3592	657.3566	3.93	159	164	(R) <u>NVLQPS(S)</u>
37	664.3311	664.3311	664.3334	-3.52	286	290	(M) <u>KMEEK(Y)</u>
38	667.3053	667.3053	667.3046	1.08	106	110	(L) <u>YAEER(Y)</u>
39	667.3053	667.3053	667.3046	1.08	107	111	(Y) <u>AEERY(P)</u>
40	667.3053	667.3053	667.3046	1.08	355	359	(V) <u>SEEFR(A)</u>
41	689.3627	689.3627	689.3651	-3.44	205	210	(E) <u>SKPVQM(M)</u>
12	725.3955	725.3955	725.3941	1.98	323	329	(K) <u>ISQAVHA(A)</u>
43	725.3955	725.3955	725.3941	1.98	370	376	(K) <u>HIATNAV(L)</u>
44	731.4355	731.4355	731.4338	2.32	229	234	(K) <u>ILELPF(A)</u>

45	733.4369	733.4369	733.4355	1.86	213	218	(Y) <u>QIGLFR</u> (V)
46	737.3843	737.3843	737.3828	1.99	185	190	(W) <u>EKAFKD</u> (E)
47	737.3843	737.3843	737.3828	1.99	186	191	(E) <u>KAFKDE</u> (D)
48	780.3903	780.3903	780.3886	2.12	104	109	(S) <u>RLYAEER</u> (R)
49	780.3903	780.3903	780.3886	2.12	105	110	(R) <u>LYAEER</u> (Y)
50	809.4678	809.4678	809.4668	1.19	375	381	(N) <u>AVLFFGR</u> (C)
51	837.4115	837.4115	837.4101	1.66	354	360	(S) <u>VSEEFRA</u> (D)
52	853.4066	853.4066	853.405	1.85	353	359	(A) <u>SVSEEFR</u> (A)
53	944.5426	944.5426	944.5411	1.56	85	92	(R) <u>DILNQITK</u> (P)
54	979.5115	979.5115	979.5095	2.05	256	263	(L) <u>ESIINF EK</u> (L)
55	985.5482	985.5482	985.5465	1.68	370	378	(K) <u>HIATNAVLF</u> (F)
56	1002.6357	1002.636	1002.635	1.07	173	181	(M) <u>VLVN AIVFK</u> (G)

57	1024.4601	1024.46	1024.462	-1.42	164	173	(P) <u>SSVDSQTAMV</u> (L)
58	1024.4601	1024.46	1024.458	1.87	187	195	(K) <u>AFKDEDTQA</u> (M)
59	1033.4944	1033.494	1033.495	-0.484	148	156	(S) <u>WVESQTNGI</u> (I)
60	1052.4694	1052.469	1052.468	1.62	268	276	(W) <u>TSSNVMEER</u> (K)
61	1066.5172	1066.517	1066.52	-2.99	260	267	(I) <u>NFEKLTEW</u> (T)
62	1066.5172	1066.517	1066.516	0.784	351	360	(D) <u>AASVSEEFRA</u> (D)
63	1067.5203	1067.52	1067.523	-2.39	330	339	(A) <u>AHAEINEAGR</u> (E)
64	1091.5957	1091.596	1091.594	1.3	312	322	(N) <u>LSGISSAESLK</u> (I)
65	1091.5957	1091.596	1091.594	1.3	313	323	(L) <u>SGISSAESLKI</u> (S)
66	1091.5957	1091.596	1091.594	1.3	314	324	(S) <u>GISSAESLKIS</u> (Q)
67	1138.5498	1138.55	1138.548	1.35	294	304	(L) <u>TSVLMAMGITD</u> (V)
68	1178.6144	1178.614	1178.613	1.55	229	239	(K) <u>ILELPFASGTM</u> (S)

69	1236.559	1236.559	1236.557	2.01	62	73	(K) <u>LPGFGDSIEAQC(G)</u>
70	1247.6257	1247.626	1247.63	-3.46	166	177	(S) <u>VDSQTAMVLVNA(I)</u>
71	1265.6474	1265.647	1265.645	2.21	229	240	(K) <u>ILELPFASGTMS(M)</u>
72	1266.6504	1266.65	1266.651	-0.54	120	130	(Q) <u>CVKELYRGGLE(P)</u>
73	1308.6455	1308.646	1308.647	-1.18	108	117	(A) <u>EERYPILPEY(L)</u>
74	1345.7376	1345.738	1345.738	0.0513	370	381	(K) <u>HIATNAVLFFGR(C)</u>
75	1396.6884	1396.688	1396.685	2.37	229	241	(K) <u>ILELPFASGTMSM(L)</u>
76	1396.6884	1396.688	1396.685	2.37	230	242	(I) <u>LELPFASGTMSML(V)</u>
77	1581.7241	1581.724	1581.721	1.74	264	276	(K) <u>LTEWTSSNVMEER(K)</u>
78	1005.4796 ⁺²	2009.952	2009.952	-0.181	331	350	(A) <u>HAEINEAGREVVGSAEAGVD(A)</u>
79	1011.5159 ⁺²	2022.025	2022.029	-2.25	122	139	(V) <u>KELYRGGLEPINFQTAAD(Q)</u>
80	1020.0315 ⁺²	2039.056	2039.059	-1.61	36	54	(M) <u>SALAMVYLGAKDSTRTQIN(K)</u>

81	1125.8968 ⁺³	3375.676	3375.675	0.247	58	88	(V) <u>RFDKL...SSLRDILN(Q)</u>
82	1138.9221 ⁺³	3414.752	3414.761	-2.82	254	281	(E) <u>QLESII...SSNVMEERKIKVY(L)</u>
83	1140.1008 ⁺²	2279.194	2279.185	3.97	118	137	(Y) <u>LQCV...YRGGLEPINFQTA(A)</u>
84	1141.0977 ⁺²	2281.188	2281.182	2.56	84	103	(L) <u>RDILN...TKPNDVYSFSLAS(R)</u>
85	1141.0977 ⁺²	2281.188	2281.182	2.56	85	104	(R) <u>DILNQITKPNDVYSFSLASR(L)</u>
86	1141.5973 ⁺²	2282.187	2282.192	-2.11	73	93	(Q) <u>CGTSVNVHSSLRDILNQITKP(N)</u>
87	1141.5973 ⁺²	2282.187	2282.187	-0.0476	305	327	(D) <u>VFSSSANLSGISSAESLKISQAV(H)</u>
88	1143.0793 ⁺²	2285.151	2285.152	-0.398	108	125	(A) <u>EERYPILPEYLQCVKELY(R)</u>
89	1143.0793 ⁺²	2285.151	2285.152	-0.493	282	300	(Y) <u>LPRMKMEEKYNLTSVLMAM(G)</u>
90	1143.5776 ⁺²	2286.148	2286.144	1.57	204	223	(Q) <u>ESKPVQMMYQIGLFRVASMA(S)</u>
91	1143.5776 ⁺²	2286.148	2286.144	1.57	206	225	(S) <u>KPVQMMYQIGLFRVASMASE(K)</u>
92	1143.5776 ⁺²	2286.148	2286.144	1.57	207	226	(K) <u>PVQMMYQIGLFRVASMASEK(M)</u>

93	1178.9364 ⁺³	3534.795	3534.792	0.637	241	271	(S) <u>MLVLL...GLEQLESIINFEKLTEWTSSN(V)</u>
94	1178.9364 ⁺³	3534.795	3534.807	-3.49	290	323	(E) <u>KYNLT...ITDVFSSSANLSGISSAESLKI(S)</u>
95	1209.6256 ⁺³	3626.862	3626.856	1.68	129	160	(G) <u>LEPINF...ARELINSWVESQTNGIIRNV(L)</u>
96	1209.6256 ⁺³	3626.862	3626.856	1.68	130	161	(L) <u>EPINFQ...AELINSWVESQTNGIIRNV(L)</u>
97	1240.9720 ⁺³	3720.901	3720.893	2.31	240	272	(M) <u>SMLVL...EQLESIINFEKLTEWTSSNV(M)</u>
98	1241.3060 ⁺³	3721.903	3721.899	1.24	224	257	(A) <u>SEKMK...MSMLVLLPDEVSGLEQLES(I)</u>
99	1266.6335 ⁺⁴	5063.512	5063.507	1.05	322	369	(L) <u>KISQAV...AASVSEEFRA DHPFLFCIK(H)</u>
100	1266.6335 ⁺⁴	5063.512	5063.5	2.48	333	379	(A) <u>EINEAGR...DHPFLFCIKHIATNAVLFF(G)</u>
101	1288.3465 ⁺³	3863.025	3863.011	3.65	228	262	(M) <u>KILELPF...LLPDEVSGLEQLESIINFE(K)</u>
102	1288.3465 ⁺³	3863.025	3863.011	3.65	229	263	(K) <u>ILELPFA...LPDEVSGLEQLESIINFEK(L)</u>
103	1288.3465 ⁺³	3863.025	3863.011	3.65	230	264	(I) <u>LELPFAS...LPDEVSGLEQLESIINFEKL(T)</u>
104	1307.6863 ⁺³	3921.044	3921.051	-1.67	275	307	(E) <u>ERKIKV...EKYNLTSVLMAMGITDVFS(S)</u>

105	1381.0551 ⁺³	4141.151	4141.15	0.207	56	93	(K) <u>VVRFD...GTSVNVHSSLRDILNQITKP(N)</u>
106	1395.9673 ⁺⁴	5580.847	5580.833	2.54	196	244	(A) <u>MPFRV...EKMKILELPFASGTMSMLVL(L)</u>
107	1395.9673 ⁺⁴	5580.847	5580.868	-3.67	243	289	(L) <u>VLLPDE...VMEERKIKVYLPRMKMEE(K)</u>
108	1412.3903 ⁺³	4235.156	4235.141	3.74	269	304	(T) <u>SSNVME...MEEKYNLTSVLMAMGITD(V)</u>
109	1521.7950 ⁺³	4563.37	4563.383	-2.8	218	259	(F) <u>RVASMA...SMLVLLPDEVSGLEQLESII(N)</u>
110	1522.7897 ⁺³	4566.355	4566.362	-1.65	138	179	(A) <u>ADQARE...VQPSSVDSQTAMVLVNAIV(F)</u>
111	1522.7897 ⁺³	4566.355	4566.369	-3.12	243	281	(L) <u>VLLPDEV...KLTEWTSSNVMEERKIKVY(L)</u>
112	1688.3410 ⁺⁶	10125.01	10125	1.01	288	383	(M) <u>EEKYNLT...LFCIKHIATNAVLFFGRCV(S)</u>
113	1689.3431 ⁺⁶	10131.02	10131.06	-3.7	261	354	(N) <u>FEKLTEW...AGREVVGSAEAGVDAASV(S)</u>
114	1767.9012 ⁺²	3534.795	3534.792	0.772	241	271	(S) <u>MLVLLPD...LEQLESIINFELTEWTSSN(V)</u>
115	1767.9012 ⁺²	3534.795	3534.807	-3.36	290	323	(E) <u>KYNLTSV...TDVFSSSANLSGISSAESLKI(S)</u>
116	363.2008 ⁺²	725.3943	725.3941	0.357	323	329	(K) <u>ISQAVHA(A)</u>

117	363.2008 ⁺²	725.3943	725.3941	0.357	370	376	(K) <u>HIATNAV(L)</u>
118	367.2216 ⁺²	733.4359	733.4355	0.529	213	218	(Y) <u>QIGLFR(V)</u>
119	369.1952 ⁺²	737.3831	737.3828	0.397	185	190	(W) <u>EKA FKD(E)</u>
120	369.1952 ⁺²	737.3831	737.3828	0.397	186	191	(E) <u>KAFKDE(D)</u>
121	388.2450 ⁺²	775.4827	775.4825	0.307	214	220	(Q) <u>IGLFRVA(S)</u>
122	388.2450 ⁺²	775.4827	775.4825	0.307	279	284	(I) <u>KVY LPR(M)</u>
123	416.5468 ⁺³	1247.626	1247.63	-3.34	166	177	(S) <u>VDSQTAMVLVNA(I)</u>
124	419.2089 ⁺²	837.4105	837.4101	0.496	354	360	(S) <u>VSEEFRA(D)</u>
125	427.2072 ⁺²	853.4071	853.405	2.46	353	359	(A) <u>SVSEEFR(A)</u>
126	449.2505 ⁺³	1345.737	1345.738	-0.434	370	381	(K) <u>HIATNAV LFFGR(C)</u>
127	454.7279 ⁺²	908.4485	908.4472	1.43	198	204	(P) <u>FRVTEQE(S)</u>
128	472.7746 ⁺²	944.5419	944.5411	0.848	85	92	(R) <u>DILNQITK(P)</u>

129	472.7746 ⁺²	944.5419	944.5386	3.5	365	372	(F) <u>LFCIKHIA(T)</u>
130	479.2958 ⁺²	957.5843	957.584	0.348	50	57	(T) <u>RTQINKVV(R)</u>
131	479.2958 ⁺²	957.5843	957.584	0.348	51	58	(R) <u>TQINKVVR(F)</u>
132	481.7512 ⁺²	962.4951	962.4975	-2.51	168	176	(D) <u>SQTAMVLVN(A)</u>
133	482.5801 ⁺³	1445.726	1445.724	0.932	326	339	(Q) <u>AVHAAHAEINEAGR(E)</u>
134	490.2588 ⁺²	979.5103	979.5095	0.853	256	263	(L) <u>ESIINF EK(L)</u>
135	492.2475 ⁺²	983.4877	983.4866	1.1	40	48	(A) <u>MVYLGAKDS(T)</u>
136	493.2774 ⁺²	985.5475	985.5465	0.996	370	378	(K) <u>HIATNAVLF(F)</u>
137	504.2487 ⁺²	1007.49	1007.491	-0.358	134	142	(N) <u>FQTAADQAR(E)</u>
138	512.7346 ⁺²	1024.462	1024.462	0.363	164	173	(P) <u>SSVDSQTAMV(L)</u>
139	512.7346 ⁺²	1024.462	1024.458	3.65	187	195	(K) <u>AFKDEDTQA(M)</u>
140	514.2736 ⁺²	1027.54	1027.539	0.56	211	218	(M) <u>MYQIGLFR(V)</u>

141	514.2736 ⁺²	1027.54	1027.539	0.56	376	384	(A) <u>VLFFGRCVS</u> (P)
142	517.2503 ⁺²	1033.493	1033.495	-1.53	148	156	(S) <u>WVESQTNGI</u> (I)
143	519.2465 ⁺³	1555.725	1555.721	2.57	187	199	(K) <u>AFKDEDTQAMPFR</u> (V)
144	526.7383 ⁺²	1052.469	1052.468	1.54	268	276	(W) <u>TSSNVMEER</u> (K)
145	533.7627 ⁺²	1066.518	1066.52	-2.12	260	267	(I) <u>NFEKLTEW</u> (T)
146	533.7627 ⁺²	1066.518	1066.516	1.65	351	360	(D) <u>AASVSEEFRA</u> (D)
147	541.2734 ⁺²	1081.54	1081.542	-2.34	29	38	(F) <u>YCPIAIMSAL</u> (A)
148	541.2734 ⁺²	1081.54	1081.538	1.38	219	228	(R) <u>VASMASEKMK</u> (I)
149	546.3021 ⁺²	1091.597	1091.594	2.42	312	322	(N) <u>LSGISSAESLK</u> (I)
150	546.3021 ⁺²	1091.597	1091.594	2.42	313	323	(L) <u>SGISSAESLKI</u> (S)
151	546.3021 ⁺²	1091.597	1091.594	2.42	314	324	(S) <u>GISSAESLKIS</u> (Q)
152	546.8021 ⁺²	1092.597	1092.594	3.09	255	263	(Q) <u>LESIINF EK</u> (L)

153	546.8021 ⁺²	1092.597	1092.594	3.09	256	264	(L) <u>ESIINF</u> EKL(T)
154	546.9207 ⁺³	1638.748	1638.743	2.89	192	205	(E) <u>DTQAMPFRVTEQES</u> (K)
155	568.8059 ⁺²	1136.605	1136.605	-0.753	293	303	(N) <u>LTSVLMAMGIT</u> (D)
156	569.3070 ⁺²	1137.607	1137.609	-1.55	120	129	(Q) <u>CVKELYRGGL</u> (E)
157	572.7967 ⁺²	1144.586	1144.584	1.47	200	209	(R) <u>VTEQESKPVQ</u> (M)
158	584.8082 ⁺²	1168.609	1168.611	-1.15	34	44	(A) <u>IMSALAMVYLG</u> (A)
159	586.2999 ⁺⁴	2342.178	2342.178	-0.271	19	39	(L) <u>KVHHANENIFYCPIAIMSALA</u> (M)
160	586.2999 ⁺⁴	2342.178	2342.185	-3.06	103	121	(A) <u>SRLYAEERYPILPEYLQCV</u> (K)
162	586.2999 ⁺⁴	2342.178	2342.174	1.65	282	301	(Y) <u>LPRMKMEEKYNLTSVLMAMG</u> (I)
163	586.2999 ⁺⁴	2342.178	2342.174	1.65	283	302	(L) <u>PRMKMEEKYNLTSVLMAMGI</u> (T)
164	589.8109 ⁺²	1178.615	1178.613	1.66	229	239	(K) <u>ILELPFASGTM</u> (S)
165	591.9726 ⁺³	1773.903	1773.899	2.35	323	339	(K) <u>ISQAVHAAHAEINEAGR</u> (E)

166	600.5545 ⁺⁴	2399.196	2399.194	1.05	303	326	(I) <u>TDVFSSSANLSGISSAESLKISQA(V)</u>
167	605.2651 ⁺²	1209.523	1209.521	2.03	190	199	(K) <u>DEDTQAMPFR(V)</u>
168	610.9805 ⁺³	1830.927	1830.924	1.56	211	226	(M) <u>MYQIGLFRVASMASEK(M)</u>
169	610.9805 ⁺³	1830.927	1830.924	1.56	212	227	(M) <u>YQIGLFRVASMASEKM(K)</u>
170	618.7843 ⁺²	1236.561	1236.557	3.89	62	73	(K) <u>LPGFGDSIEAQC(G)</u>
171	620.3278 ⁺³	1858.969	1858.966	1.66	142	157	(A) <u>RELINSWVESQTNGII(R)</u>
172	620.3278 ⁺³	1858.969	1858.966	1.66	143	158	(R) <u>ELINSWVESQTNGIIR(N)</u>
173	620.3278 ⁺³	1858.969	1858.969	-0.149	156	172	(G) <u>IIRNVLPSSVDSQTAM(V)</u>
174	624.3179 ⁺²	1247.629	1247.63	-1.19	166	177	(S) <u>VDSQTAMVLVNA(I)</u>
175	633.3271 ⁺²	1265.647	1265.645	1.83	229	240	(K) <u>ILELPFASGTMS(M)</u>
176	633.8286 ⁺²	1266.65	1266.651	-0.916	120	130	(Q) <u>CVKELYRGGLE(P)</u>
177	657.6627 ⁺³	1970.974	1970.971	1.17	72	89	(A) <u>QCGTSVNVHSSLRDILNQ(I)</u>

178	664.3312 ⁺²	1327.655	1327.653	1.71	92	103	(T) <u>KPNDVYSFSLAS</u> (R)
179	666.3396 ⁺²	1331.672	1331.67	1.26	131	142	(E) <u>PINFQTAADQAR</u> (E)
180	673.3746 ⁺²	1345.742	1345.738	3.26	370	381	(K) <u>HIATNAVLFFGR</u> (C)
181	674.6792 ⁺³	2022.023	2022.029	-2.98	122	139	(V) <u>KELYRGGLEPINFQTAAD</u> (Q)
182	698.8479 ⁺²	1396.689	1396.685	2.46	229	241	(K) <u>ILELPFASGTMSM</u> (L)
183	698.8479 ⁺²	1396.689	1396.685	2.46	230	242	(I) <u>LELPFASGTMSML</u> (V)
184	703.8380 ⁺²	1406.669	1406.665	2.35	200	211	(R) <u>VTEQESKPVQMM</u> (Y)
185	716.3991 ⁺²	1431.791	1431.791	-0.345	43	55	(Y) <u>LGAKDSTRTQINK</u> (V)
186	719.3749 ⁺²	1437.743	1437.748	-3.82	227	239	(K) <u>MKILELPFASGTM</u> (S)
187	723.3666 ⁺²	1445.726	1445.724	1.05	326	339	(Q) <u>AVHAAHAEINEAGR</u> (E)
188	732.3577 ⁺²	1463.708	1463.712	-2.67	235	248	(F) <u>ASGTMSMLVLLPDE</u> (V)
189	738.8552 ⁺²	1476.703	1476.7	2.19	159	172	(R) <u>NVLQPSSVDSQTAM</u> (V)

190	761.4018 ⁺³	2282.191	2282.192	-0.567	73	93	(Q) <u>CGTSVNVHSSLRDILNQITKP(N)</u>
191	761.4018 ⁺³	2282.191	2282.187	1.5	305	327	(D) <u>VFSSSANLSGISSAESLKISQAV(H)</u>
192	763.0610 ⁺³	2287.168	2287.172	-1.43	88	107	(L) <u>NQITKPNDVYSFSLASRLYA(E)</u>
193	763.4039 ⁺²	1525.801	1525.804	-2.47	38	51	(A) <u>LAMVYLGAKDSTRT(Q)</u>
194	768.3679 ⁺²	1535.729	1535.727	0.806	8	19	(S) <u>MEFCFDVFKELK(V)</u>
195	769.3906 ⁺²	1537.774	1537.77	2.29	307	322	(F) <u>SSSANLSGISSAESLK(I)</u>
196	781.3975 ⁺³	2342.178	2342.178	-0.195	19	39	(L) <u>KVHHANENIFYCPIAIMSALA(M)</u>
197	781.3975 ⁺³	2342.178	2342.185	-2.98	103	121	(A) <u>SRLYAEERYPILPEYLQCV(K)</u>
198	781.3975 ⁺³	2342.178	2342.174	1.72	282	301	(Y) <u>LPRMKMEEKYNLTSVLMAMG(I)</u>
199	781.3975 ⁺³	2342.178	2342.174	1.72	283	302	(L) <u>PRMKMEEKYNLTSVLMAMGI(T)</u>
200	785.3696 ⁺²	1569.732	1569.729	2.03	200	212	(R) <u>VTEQESKPVQMMY(Q)</u>
201	787.0622 ⁺³	2359.172	2359.165	2.9	356	375	(S) <u>EEFRADHPFLFCIKHIATNA(V)</u>

202	791.3658 ⁺²	1581.724	1581.721	1.88	264	276	(K) <u>LTEWTSSNVMEER(K)</u>
203	813.8802 ⁺²	1626.753	1626.747	3.87	59	73	(R) <u>FDKLPFGDSIEAQC(G)</u>
204	819.8771 ⁺²	1638.747	1638.743	2.51	192	205	(E) <u>DTQAMPFRVTEQES(K)</u>
205	820.7799 ⁺³	2460.325	2460.317	3.44	159	181	(R) <u>NVLQPSSVDSQTAMVLVNAIVFK(G)</u>
206	821.4023 ⁺²	1641.797	1641.797	0.428	301	317	(M) <u>GITDVFSSSANLSGISS(A)</u>
207	839.7861 ⁺³	2517.344	2517.338	2.23	159	182	(R) <u>NVLQPSSVDSQTAMVLVNAIVFKG(L)</u>
208	844.4258 ⁺²	1687.844	1687.84	2.67	126	141	(Y) <u>RGGLEPINFQTAADQA(R)</u>
209	844.4258 ⁺²	1687.844	1687.84	2.67	127	142	(R) <u>GGLEPINFQTAADQAR(E)</u>
210	846.4208 ⁺²	1691.834	1691.839	-2.62	97	110	(V) <u>YSFSLASRLYAEER(Y)</u>
211	846.4208 ⁺²	1691.834	1691.839	-2.62	98	111	(Y) <u>SFSLASRLYAEERY(P)</u>
212	883.9546 ⁺²	1766.902	1766.896	3.39	85	99	(R) <u>DILNQITKPNDVYSF(S)</u>
213	884.9549 ⁺²	1768.903	1768.905	-1.17	227	242	(K) <u>MKILELPFASGTMSML(V)</u>

214	887.4550 ⁺²	1773.903	1773.899	2.06	323	339	(K) <u>ISQAVHAAHAEINEAGR</u> (E)
215	915.9655 ⁺²	1830.924	1830.924	-0.199	211	226	(M) <u>MYQIGLFRVASMASEK</u> (M)
216	915.9655 ⁺²	1830.924	1830.924	-0.199	212	227	(M) <u>YQIGLFRVASMASEKM</u> (K)
217	929.9892 ⁺²	1858.971	1858.966	2.89	142	157	(A) <u>RELINSWVESQTNGII</u> (R)
218	929.9892 ⁺²	1858.971	1858.966	2.89	143	158	(R) <u>ELINSWVESQTNGIIR</u> (N)
219	929.9892 ⁺²	1858.971	1858.969	1.08	156	172	(G) <u>IIRNVLPSSVDSQTAM</u> (V)
220	929.9892 ⁺²	1858.971	1858.977	-3.15	321	338	(S) <u>LKISQAVHAAHAEINEAG</u> (R)
221	934.4169 ⁺²	1867.827	1867.83	-2.1	343	361	(V) <u>GSAEAGVDAASVSEEFRAD</u> (H)
222	951.4862 ⁺²	1901.965	1901.964	0.747	159	176	(R) <u>NVLPSSVDSQTAMVLVN</u> (A)
223	984.0115 ⁺²	1967.016	1967.012	1.88	85	101	(R) <u>DILNQITKPNDVYSFSL</u> (A)
224	985.9907 ⁺²	1970.974	1970.971	1.46	72	89	(A) <u>QCGTSVNVHSSLRDILNQ</u> (I)

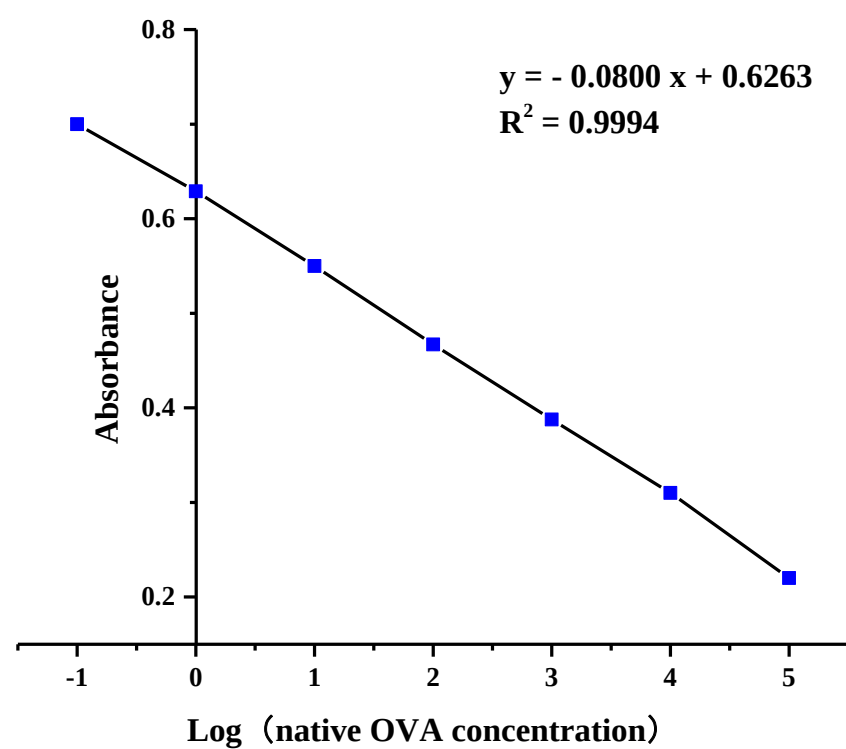


Fig. S1 Standard curve for calculating IgG binding ability.

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