

Effect of Covalent Interaction with Chlorogenic Acid on the Allergenic Capacity of Ovalbumin

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ABSTRACT: Ovalbumin (OVA) is a major allergen in avian egg white. Here, we investigated the conjugation of OVA and chlorogenic acid (CHA) to reduce the allergenic capacity of OVA. OVA–CHA conjugate was characterized by SDS-PAGE, MALDI-TOF-MS, differential scanning calorimetry, and multispectroscopic methods. Sites of the OVA–CHA conjugate were identified by LC-MS/MS. CHA possibly conjugated with Lys20 and Lys17 in OVA, which resulted in the unfolding of OVA. ELISA and Western blot assay indicated that the OVA–CHA conjugate reduced the IgE binding capacity of OVA. The results also indicated that the ability of the OVA–CHA conjugate to activate histamine release was reduced. The decreased allergenic capacity of OVA was attributed to changes in the protein structure. Moreover, the CHA binding site in OVA might directly shield the linear IgE epitope, thereby reducing the IgE binding ability. Also, the OVA–CHA conjugate showed high antioxidant activity. OVA conjugated with CHA may be a promising method of OVA hyposensitization.

KEYWORDS: chlorogenic acid, ovalbumin, conjugation, allergenicity, site of conjugation

1. INTRODUCTION

Polyphenols that form complexes with food proteins have been extensively studied,¹ which can be used to improve the functional attributes of the protein,² for application in the food industry.³ Polyphenol–protein complexes may be noncovalent or covalent, which can affect obviously the function and structure of the protein.⁴

Recently, some studies showed that some major food allergens could reduce their IgE-binding capacity by non-covalent or covalent interactions.⁵ The interaction of the major cherry allergen Pru av 1 with quercetin, gallic acid, and epicatechin reduced its IgE-binding capacity.⁶ A similar noncovalent interaction with polyphenols reduced the IgE-binding capacity of peanuts and β -lactoglobulin.^{7,8} Compared with noncovalent linking, covalently linking polyphenol to protein can generate a more stable conjugate, which is more suitable for food processing.⁹ We previously reported that covalent conjugation of β -lactoglobulin with dietary polyphenols might reduce the allergenic capacity of β -lactoglobulin.⁵ To optimize the use of polyphenol–protein interactions for reducing the allergenicity of food allergen, we need a better understanding of covalent interactions between the food allergen and polyphenol.

Ovalbumin (OVA) is the predominant protein in egg white,¹⁰ which is a major allergen in egg white, causing allergic reactions particularly in infants and young children.^{11,12} A number of studies have investigated the use of food-processing methods for reducing OVA allergenicity, such as hydrolysis, heating, and high-pressure treatments. However, a major impediment to reduce OVA allergenicity is the risk to alter the functional attributes of OVA.¹³ OVA was able to form complexes by noncovalent interaction with apple polyphenols to reduce specific antibody binding.¹⁴ (–)-Epigallocatechin gallate (EGCG) could bind OVA also by noncovalent interaction but did not prevent the IgE binding.¹⁵ To our

knowledge, the effect of covalent interactions with polyphenols on the allergenic capacity of OVA is unknown. Furthermore, the details of covalent interactions between polyphenols and OVA, especially for conjugation sites, have not been elucidated.

This research investigated the effect of covalent interactions with chlorogenic acid (CHA) on the allergenic capacity of OVA and determined the impact of this interaction on the structure and antioxidant activity of OVA.

2. MATERIALS AND METHODS

2.1. Materials. CHA (purity > 99%), OVA (protein content > 98%), 2,2-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid (ABTS), Folin-Ciocalteu phenol reagent, 1,10-diphenyl-2-picrylhydrazyl (DPPH), 3,3'-5,5'-tetramethylbenzidine (TMB), and trypsin were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). HRP-conjugated goat antihuman IgE was purchased from Kirkegaard & Perry Laboratories (Gaithersburg, MD, USA). Mouse IgG-antihuman IgE antibody and NeutrAvidin-HRP-conjugated antibody were from SouthernBiotech (Birmingham, AL, USA). All other chemicals were of analytical grade.

2.2. Sera. Sera for the ELISA and Western blot analysis were by kindly provided by Shenzhen Children's Hospital (SZCH). Pooled human serum samples were collected from 12 children allergic to chicken egg with IgE levels to chicken egg above 10 kU/L. Sera from individuals ($n = 5$) with no allergy to chicken egg were used as negative controls.

2.3. Sample Preparation. The OVA–CHA conjugate was synthesized by the free radical method according to the literature⁵ with some modification. Briefly, 0.2 g of OVA was dissolved in 50 mL of distilled H₂O. Then 1 mL of 5.0 M H₂O₂ containing 0.25 g of ascorbic acid was added. After 2 h, 0.08 g of CHA was added and

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continuously stirred for 24 h. The sample was dialyzed at 4 °C for 48 h against Milli-Q water to remove the unreacted CHA. The vaporized solid was obtained by lyophilization. The control OVA was treated in the same way with the absence of CHA.

2.4. Physicochemical Characterization of the OVA–CHA Conjugate.

2.4.1. Measurement of the Amount of CHA of the OVA–CHA Conjugate. The CHA content in the OVA–CHA conjugate was measured according to our previous paper⁵ by the Foline–Ciocalteu method using CHA as an individual standard. A 2.5 mL amount of freshly prepared Foline–Ciocalteu phenol reagent (0.2 M) was mixed thoroughly with 0.5 mL of sample (1 mg/mL) at 25 °C. After 5 min, 2 mL of sodium carbonate solution (7.5%, w/v) was added, and the mixture was placed in the dark for 2 h with intermittent shaking at 25 °C. Finally, the absorbance of the mixture at 760 nm was measured. The amount of CHA in the sample was calculated according to a standard calibration curve of CHA.

2.4.2. SDS-PAGE. SDS-PAGE was carried out on a 12% separating gel and 5% stacking gel. After heating at 95 °C for 5 min, the solutions of OVA, control OVA, and OVA–CHA conjugate (1 mg/mL) were diluted in a ratio of 1:1 loading buffer. The loading volume of samples was 15 μ L. The gels were stained with Coomassie Brilliant Blue R250. Finally, the gels were destained with deionized water.

2.4.3. MALDI-TOF MS. MALDI-TOF MS was used to measure the molecular weights of OVA and OVA–CHA conjugate according to a previous report⁵ with modification. In brief, a 1 mg amount of sample was dissolved in 1 mL of 0.1% acetonitrile/trifluoroacetic acid (50%, v/v) containing 2 mg of DTT. A 0.5 μ L amount of this solution was placed on the target. Then a 0.5 μ L amount of 2,5-dihydroxyacetophenone as a matrix solution (18 mg/mL diammonium hydrogen citrate dissolved in 125 μ L of water and 7.6 mg of 2,5-DHAP dissolved in 375 μ L of ethanol) was added to cover the target. The samples were dried in air prior to measurements. The mass spectra were recorded with a TM MALDI-TOF/TOF (Bruker Daltonics, Billerica, MA, USA).

2.5. Structural Analysis of the OVA–CHA Conjugate.

2.5.1. Fluorescence Measurements. Fluorescence measurements were performed on a Hitachi-850 spectrofluorometer (Hitachi Co., Tokyo, Japan) at 25 °C. Samples were dissolved in phosphate-buffered saline (PBS) (pH 7.2), and the protein concentration was 0.1 mg/mL. The emission was recorded in the range of 300–500 nm using an excitation wavelength of 295 nm.

2.5.2. Fourier Transform Infrared (FTIR) Spectroscopic Measurements. The FTIR spectra of the OVA, control OVA, and OVA–CHA conjugate were obtained by a FTIR-8300PCS spectrometer (SHIMADZU Co., Kyoto, Japan) using KBr pellets at 25 °C. The samples were measured from 400 to 4000 cm^{-1} with 4 cm^{-1} resolution and 32 scans. KBr was used as a reference. The quantitative analysis of the protein secondary was determined according to our previous report.¹⁶ In brief, Fourier self-deconvolution and secondary derivative were applied in the range of 1700–1600 cm^{-1} .

2.5.3. Circular Dichroism (CD). CD measurements were performed on a Jasco-810 spectrophotometer (Jasco Co., Japan) at 25 °C using a 1.0 mm path length. Samples were dissolved in PBS (pH 7.2), and the protein concentration was 0.015 mg/mL. The ellipticity was measured in the range of 190–250 nm with a 100 nm/min speed step and resolution of 0.2 nm. The collected data were uploaded to DichroWeb to analyze the secondary structure of the protein.

2.5.4. Measurement of Thermal Stability. The thermal stability of the OVA, control OVA, and OVA–CHA conjugate was examined by the differential scanning calorimetry (DSC) method using a DSC-60 calorimeter (Shimadzu, Tokyo, Japan). A 5 mg amount of sample was sealed in an aluminum pan tightly. Using nitrogen as the transfer gas at a flow rate of 30 mL/min, the thermal analyses were performed from 30 to 180 °C at a constant rate of 5 °C/min.

2.6. Identification of Sites of OVA–CHA Conjugate.

2.6.1. LC-MS/MS. To study the sites of OVA–CHA conjugate, LC-MS/MS was applied. The samples in excised gel pieces from the above SDS-PAGE were digested by trypsin as described.¹⁷ The lyophilized tryptic peptides were resuspended in 2% acetonitrile containing 0.1% formic acid. A 4 μ L amount of this solution was

injected onto a ChromXP C18 (120 Å, 3 μ m) trap column. The Eksigent nano LC 415 system (SCIEX, Concord, ON, Canada) was applied to the online chromatography separation. The trapping and desalting procedure was performed at a flow rate of 5 μ L/min with solvent (acetonitrile/H₂O/formic acid [2/98/0.1%; B, 98/2/0.1%]). Then an elution gradient of 8–38% solvent B was used to elute the peptides in 20 min. A Triple TOF 6600 mass spectrometer (Sciex, Concord, ON, Canada) was used to identify the peptides. TOF-MS scans were obtained from 350 to 1500 m/z . Data were acquired with an interface heater temperature of 150 °C, nebulizer gas of 12 psi, an ion spray voltage of 2.4 kV, and curtain gas of 35 psi. The MS data were collected using information-dependent acquired mode (IDA). TOF-MS scan accumulation time was set at 250 ms. In each scan cycle the precursor ion was selected to perform product ion scan if intensity was more than 160 cps and had a charge between +2 and +4. A maximum of 30 parent ions was allowed, and each product ion scan time was 60 ms. A rolling CE (collision energy) was applied to all precursor ions for CID fragmentation. Dynamic exclusion was set at 6 s, and MS/MS scan mass range was from 100 to 1500 m/z .

2.6.2. Database Search for Identification of Modification Sites.

The acquired MS/MS data were submitted to ProteinPilot Software v4.5 (Sciex, Concord, ON, Canada) to search against a protein database and generate Mascot generic format (.mgf) files for further protein identification by the Mascot search engine. Mascot was set up to search the uniprot protein database with the parameters as below: enzyme, trypsin; mass type, monoisotopic; max missed cleavages, 2; peptide mass tolerance, ± 15 ppm; fragment mass tolerance, ± 0.1 Da; type of search, MS/MS ion search; no fixed modifications; variable modifications, Gln > pyro-Glu(N-term Q), oxidation(M), deamidated (NQ), and defined modification (a formula of C₁₆H₁₈O₉ with specificities of cysteine/lysine/histidine/methionine/tryptophan/tyrosine). For the Modified peptides with defined modification, the mascot ion score should be ≥ 10 , and then the corresponding spectra were further manually confirmed to meet the following standard: peptide spectrum match (PSM) > 2 with at least 3 consecutive b or y ions.

2.7. Allergenic Capacity of OVA–CHA Conjugate.

2.7.1. ELISA Experiments. Competitive ELISA was used to evaluate the IgE-binding inhibition as we previously described¹⁸ with modification. In brief, 100 μ L of sample solution (2 mg/mL) was added to the wells of a plate and incubated for 16 h at 4 °C. In total, 50 μ L of concentrations of samples (0.01–100 μ g/mL) was mixed with 50 μ L of egg-allergic patient sera (diluted 1:200). The mixture was added to each well and incubated for 1 h. Then 100 μ L of HRP-conjugated goat antihuman IgE antibody (1:10 000) was added. After incubation for 40 min, 50 μ L of substrate (5 μ L of H₂O₂ and 0.4 mg/mL *o*-phenylenediamine) was added. Finally, 50 μ L of H₂SO₄ (2 M) was added to stop the enzyme reaction. The absorbance at 450 nm was measured.

2.7.2. Western Blot Analysis. Specific IgE binding capability was also analyzed by Western blot analysis as previously described.⁵ Briefly, after SDS-PAGE, proteins were transferred to polyvinylidene fluoride membranes. Then the membranes were blocked with 1% fish gelatin. After incubation for 2 h, the membranes were incubated with egg-allergic patient sera (diluted 1:50) for 1 h. Mouse IgG-antihuman IgE antibody was added to the membranes. After incubation, the membranes were incubated with NeutrAvidin-HRP-conjugated antibody (1:2000) for 1 h. Finally, the protein bands were detected using ECL Analysis System (GE Healthcare, Buckinghamshire, UK).

2.7.3. Histamine Release Experiments. Histamine release was measured as previously described.¹⁹ Briefly, peripheral blood mononuclear cells were isolated from a nonallergic individual. These cells were treated by lactic acid to strip their unspecific IgE. Basophils were resensitized with IgE from egg-allergic patient serum and then stimulated with native OVA, control OVA, and OVA–CHA (0.01–10 μ g/mL). Bovine serum albumin (BSA; 10 μ g/mL) was used as a negative control. Antigen-induced histamine release involved use of a competitive enzyme immunoassay histamine kit (Immuno-tech, Marseille, France) according to the instructions from the manufacturer. After subtraction of the spontaneous released

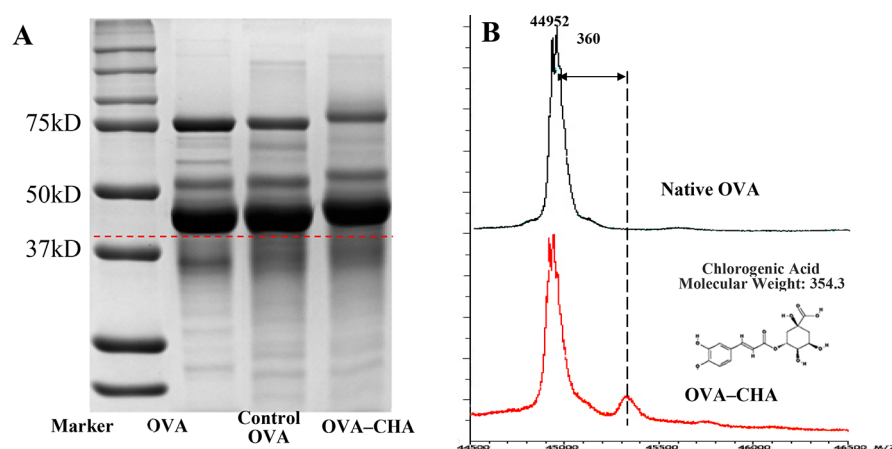


Figure 1. SDS-PAGE (A) and MOLDI-TOF-MS (B) spectra for OVA, control OVA, and OVA-CHA conjugate.

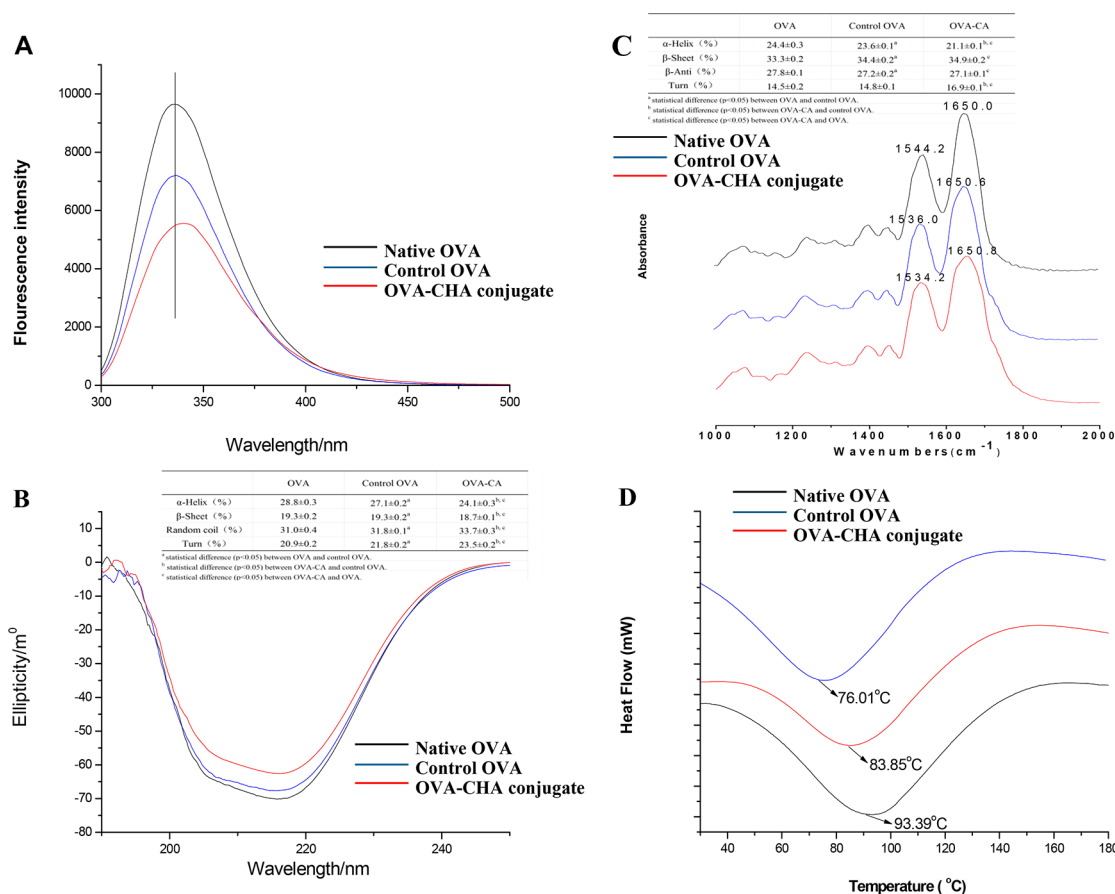


Figure 2. Fluorescence (A), CD (B), FTIR (C), and DSC (D) spectra for OVA, control OVA, and OVA-CHA conjugate. Related secondary structure contents analyzed by DichroWeb and FTIR are summarized in the inset in B and C, respectively.

histamine, the released histamine was calculated as percentage of the total released histamine. All experiments were measured in triplicate on separate days.

2.8. Antioxidant Activity Analysis of OVA-CHA Conjugate.

2.8.1. DPPH Scavenging Activity. DPPH scavenging activity of the OVA, control OVA, and OVA-CHA conjugate was measured according to the previous report.²⁰ In brief, 2 mL of DPPH was mixed with 2 mL of sample (0.5 mg/mL). The mixture was stored in the dark for 1 h. Finally, the absorbance at 517 nm was measured.

2.8.2. ABTS⁺ Scavenging Activity. ABTS⁺ scavenging activity of the OVA, control OVA, and OVA-CHA conjugate was performed according to the method of a previous report.²¹ In brief, 3 mL of ABTS reagent was mixed with 1 mL of sample (0.5 mg/mL). The

mixture was incubated for 1 h at 25 °C. Finally, the absorbance at 734 nm was measured.

2.9. Statistical Analysis. In this study, all sample determinations were carried out in triplicate. The results were expressed as mean $\pm$ standard deviation. ANOVA and Duncan least significant difference tests were used to determine statistical differences between groups. Differences with a p value < 0.05 were statistically significant.

3. RESULTS AND DISCUSSION

3.1. Physicochemical Characterization of the OVA-CHA Conjugate. SDS-PAGE was used to analyze the molecular weight change of the OVA-CHA conjugate (Figure

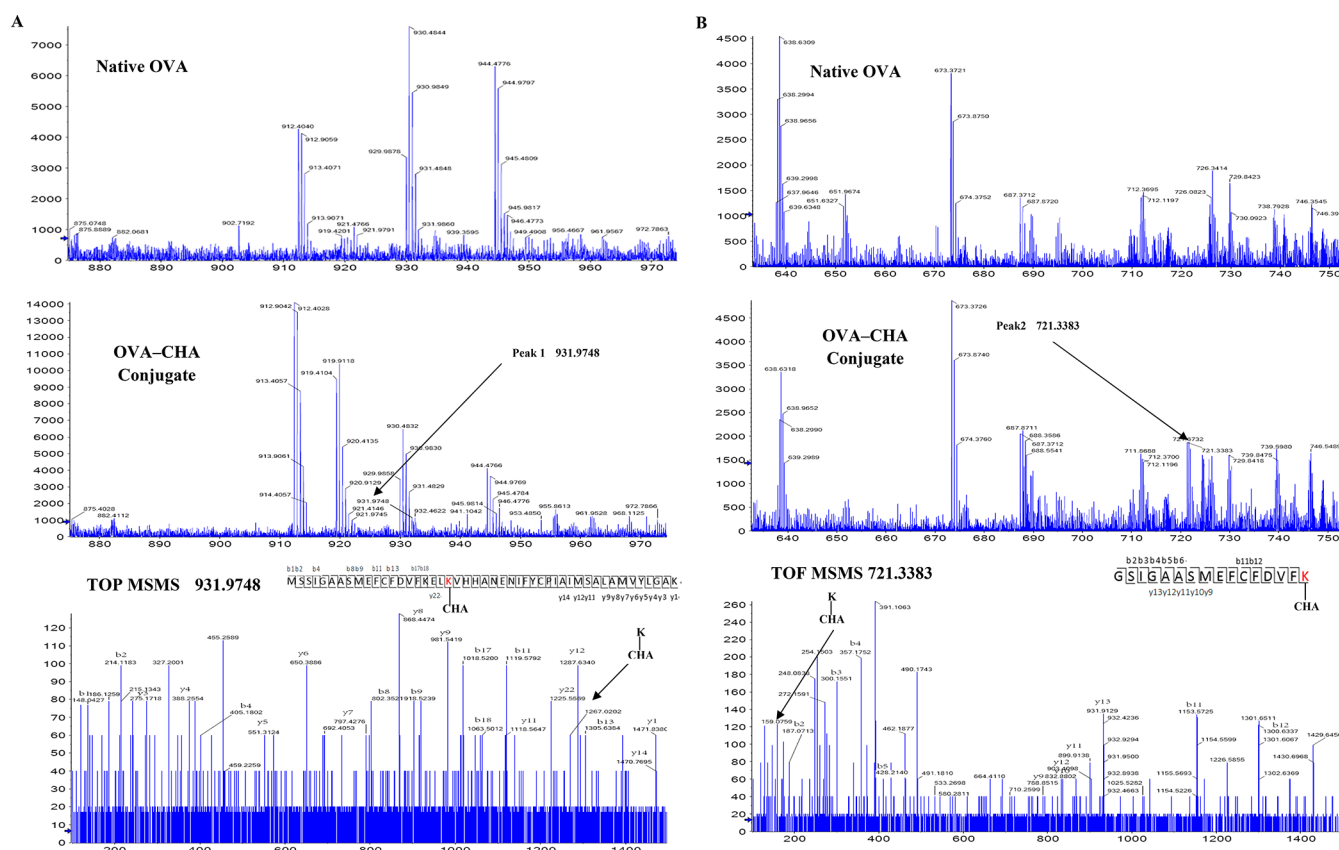


Figure 3. LC-MS spectra of OVA and OVA-CHA conjugate; interpretation of LC-MS/MS spectra of peak 1 at m/z 931.9748 (A) and peak 2 at m/z 721.3383 (B).

1A). The main band in OVA and the OVA control was about 45 kDa. The band of the OVA-CHA conjugate migrated up, which suggested that conjugation with CHA increased the molecular weight of OVA. The change in molecular weight of the OVA-CHA conjugate was further evaluated by MALDI-TOF-MS (Figure 1B). The molecular weight of OVA was about 45 kDa. The molecular weight of the OVA-CHA conjugate was increased, as shown by a new peak. The difference in molecular weight (~360 Da) between OVA and the OVA-CHA conjugate might due to the conjugated CHA molecule into the protein. This result agreed with the SDS-PAGE finding. The CHA content of the OVA-CHA conjugate was 14.8 ± 1.4 mg.

3.2. Structural Analysis of the OVA-CHA Conjugate.

The Trp fluorescence study helped to obtain the information about the conformational changes of the proteins. As shown in Figure 2A, the fluorescence emission maximum wavelength for the OVA was 334.2 nm, and the emission maxima of the control OVA showed a slight red shift (from 334.2 to 334.6 nm) with an insignificant lower fluorescence intensity. As compared with OVA, the emission maxima of the OVA-CHA conjugate showed a remarkable red shift (from 334.2 to 340.4 nm) with lower fluorescence intensity, which was related to more exposure of Trp residues. Feng et al. also observed a similar result of conjugates of OVA with catechins.²² This result suggested that conjugation with CHA resulted in the unfolding protein structure of OVA.²³

CD spectra of OVA, control OVA, and OVA-CHA conjugate are in Figure 2B. The CD spectra for OVA showed a broad negative band (210–220 nm). The control OVA and OVA-CHA conjugate showed a slight decrease and an

obvious decrease in the absolute θ values of this negative band, respectively, indicating the loss of some secondary structures in the protein after conjugation with CHA.

The Selcon3 method in DichroWeb online was used to evaluate the secondary structure. In this study, OVA contained 19.3% β -sheet, 28.8% α -helix, 31.0% β -turn, and 20.9% unordered element content. In general, free radical treatment led to a slight decrease in the α -helix fraction with a parallel increase in unfolded structure content, but the content of β -sheets hardly changed; the conjugation with CHA decreased α -helix and β -sheet content with a parallel increase in the unfolded structure content.

FTIR analysis was used to further study the conformational changes of OVA due to conjugation with CHA. The FTIR spectra from 1000 to 2000 cm^{-1} are shown in Figure 2C. The spectra of the native OVA showed the amide I and amide II peak positions at 1650.0 and 1544.2 cm^{-1} , respectively. For the control OVA, the amide I moved to 1650.6 cm^{-1} and amide II moved to 1536.0 cm^{-1} , suggesting that the free radical treatment altered the N-H or C-N bond of OVA; for the conjugate, the amide I moved to 1650.8 cm^{-1} and amide II moved to 1534.2 cm^{-1} , suggesting conjugation with CHA changed the secondary structure of the OVA.

The curve-fitting method was applied in the range from 1600 to 1700 cm^{-1} to calculate the protein secondary structure content. In this study, OVA contained 24.4% α -helix, 33.3% β -sheet, 27.8% β -anti, and 14.5% turn content. OVA conjugation with CHA decreased α -helix content with a parallel increase in β -sheet content. This trend differed from CD results. This difference may due to the method of sample preparation because aqueous solution was used for CD spectroscopy but

lyophilized powder for FTIR. Furthermore, the conformational change of OVA was not great, and the CD and FTIR spectra used to determine secondary structures are not very accurate.²³

The conjugate was also thermally characterized by DSC (Figure 2D). The OVA, control OVA, and OVA-CHA conjugate showed thermal denaturation temperatures at 93.39, 83.85, and 76.01 °C, respectively. The thermal stability of the protein was associated with the protein structure. These results of thermal stability are consistent with CD and fluorescence findings. Thus, the decreased denaturation temperature of OVA was attributed to conjugation with CHA. Feng et al. had similar observations: conjugation with catechins decreased the denaturation temperature of OVA.²²

The results of the structural analysis suggested that the free radical treatment used in this study appeared to induce changes in the protein, and the covalent conjugation of CHA decreased the tertiary conformation stability of OVA.

3.3. Identification of Sites of OVA-CHA Conjugate.

We performed LC-MS/MS for the OVA and OVA-CHA conjugate using trypsin digestion. As shown in Figure 3, two new peaks were identified for the conjugate at m/z 931.9748 (Figure 3A) and m/z 721.3383 (Figure 3B), which could be the conjugation locations. The MS/MS spectra for these two ions are shown in the figure. MS/MS data for the charged ion at m/z 931.9748 and 721.3383 were identified as MSSIGAAS-MEFCFDVFKELK-chlorogenic acidVHHANENIFYCPIAIM-SALAMVYLGAK (residues 1–47) and GSIGAAS-MEFCFDVFK-chlorogenic acid (residues 2–17), respectively. Thus, CHA possibly conjugated with Lys20 and Lys17 in the OVA.

Protein-polyphenol conjugate can be prepared by the alkaline or free radical method. Several studies reported that phenolic compounds are oxidized to their respective quinones in the presence of alkaline. These quinones could react with nucleophilic groups in the protein chains, such as tyrosine, lysine, histidine, tryptophan, and cysteine residues.^{24,25} Prigent et al. found that in covalent interactions between proteins and caffeoylquinic acid the chains of the protein were more reactive to lysine and tyrosine than tryptophan and histidine.²⁶ In this work, the conjugate was prepared by using the radical. According to previous reports,^{2,27} the hydroxyl radicals generated from the reaction between redox pair components might attack the amino acid-like lysine in OVA, which generate radical species. Then the CHA react with these radical species of the protein, forming covalent bonds. Thus, in this work, the Lys20 and Lys17 in OVA changed to radical species and interacted with CHA, generating the OVA-CHA conjugate.

3.4. Allergic Capacity of OVA-CHA Conjugate. The IgE-binding capacity of the OVA, control OVA, and OVA-CHA conjugate was analyzed by ELISA (Figure 4A). The OVA-CHA conjugate showed a lower IgE inhibition curve than the native OVA and control OVA, suggesting the IgE binding to the OVA was apparently decreased after conjugation with CHA.

To further evaluate the IgE-binding capacity, Western blot analysis was performed. The bands of native OVA, control OVA, and OVA-CHA conjugate were strongly recognized by IgE, and the density of the band did not differ between the control OVA and the native OVA (Figure 4B). However, band density was reduced for the OVA-CHA conjugate as compared with the native and control OVA. This result again indicated that CHA conjugation with OVA could inhibit IgE binding.

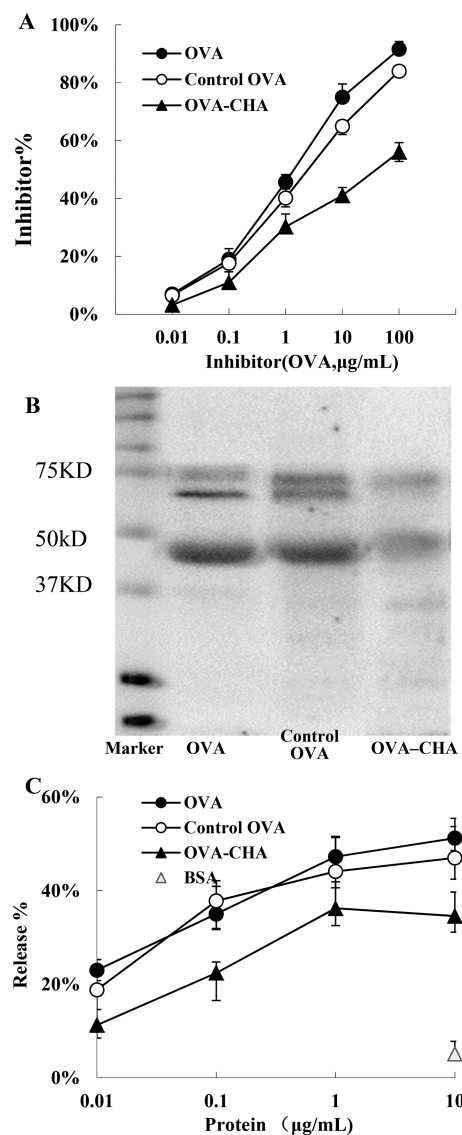


Figure 4. ELISA (A), Western blot analysis (B), and mean basophil histamine release (C) of OVA, control OVA, and OVA-CHA conjugate.

Basophils from nonallergic donors were sensitized with IgE present in sera (Figure 4C). In general, OVA-CHA conjugate showed less biological activity than native OVA and control OVA in the basophil histamine release assay. At 10 µg/mL, OVA stimulated about 50% of the maximum histamine release, whereas conjugate OVA-CHA stimulated only about 35%. Thus, as compared with OVA, OVA-CHA reduced the cross-link IgE binding to receptors on basophils, thereby inducing less histamine release. These results agree with IgE binding-capacity testing (Figure 4A and 4B), so conjugation with CHA could decrease the potential allergenicity of OVA.

Most studies reported that OVA allergies were mediated by IgE.²⁸ The IgE-binding capacity of OVA is directly related to epitopes, including conformational and linear epitopes. Conjugation with CHA was shown to modify OVA, thereby resulting in the unfolding of OVA. This modification might change or destroy the conformational epitopes of OVA, which affects the IgE binding to these conformational IgE epitopes. Furthermore, when CHA was located in or near linear IgE epitopes, the linear epitope was modified, which led to

Table 1. Antioxidant Activity of OVA, Control OVA, and OVA–CHACconjugate

property/sample	OVA	control OVA	OVA–CHA	P
DPPH scavenging activity ($\mu\text{mol Trolox equiv/g sample}$)	10.25 $\pm$ 0.39	10.58 $\pm$ 0.75	22.29 $\pm$ 1.10 ^{b,c}	<0.01
ABTS scavenging activity ($\mu\text{mol Trolox equiv/g sample}$)	26.66 $\pm$ 2.40	28.81 $\pm$ 1.28 ^a	66.90 $\pm$ 3.18 ^{b,c}	<0.01

^a*p* < 0.05 between OVA and control OVA. ^b*p* < 0.05 between OVA–CHA and OVA. ^c*p* < 0.05 between OVA–CHA and control OVA.

decreased IgE binding activity. Several reports of IgE-binding sites of OVA have been published, but results differ because of differences in the patient populations used in each study. Elsayed et al. reported that the peptide Phe11–Leu19 was one linear IgE epitope on OVA.²⁹ Our LC-MS/MS results suggested that CHA conjugated with Lys20 and Lys17 in OVA. Thus, the CHA binding site in OVA might directly shield the peptide Phe11–Leu19, which prevents IgE from accessing this linear IgE epitope. The decrease in allergenic capacity of OVA may be attributed to changes in the protein conformation and the shielding of the linear IgE epitope by covalent conjugation with CHA.

3.5. Antioxidant Activity of OVA–CHA Conjugate.

The antioxidant activities of the OVA, control OVA, and OVA–CHA conjugate were evaluated by DPPH and ABTS⁺ radical scavenging assay (Table 1). The DPPH and ABTS⁺ values for OVA were 10.25 $\pm$ 0.39 and 26.66 $\pm$ 2.40 $\mu\text{mol trolox equivalent/g sample}$, respectively; these values were increased 2–3 times after conjugation with CHA. These results indicated that conjugation with CHA enhanced the antioxidant activity of OVA.

OVA has been widely used as a standard model for studies of protein structure, protein functional properties, and food allergy.^{13,30} OVA would enhance the functional properties of the protein by covalent conjugation with polyphenols, such as antioxidant activities, emulsifying activity, and emulsifying stability.^{22,31,32} In this study, the antioxidant activity of OVA was improved by introducing OH groups from CHA. The OVA–CHA conjugate showed reduced allergenic capacity with high antioxidant activity, so covalent conjugation of OVA with polyphenol may be a way to reduce the allergenicity of the protein.

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Notes

The authors declare no competing financial interest.

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