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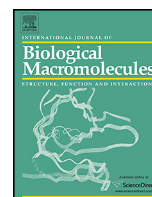

Cross-linked ovalbumin catalyzed by polyphenol oxidase: Preparation, structure and potential allergenicity

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ABSTRACT

Ovalbumin (OVA) is described as one of the major allergens in hen's egg, and it is the most abundant protein in egg white. Enzyme-mediated covalent cross-linking of food proteins, can influence their structure and allergenicity. The aim of this study was to investigate the potential of polyphenol oxidase from *Agaricus bisporus* to cross-link OVA (CL-OVA) in the presence or absence of caffeic acid, followed by characterizing the structure and allergenicity of CL-OVA. A single-factor experiment was designed to assess the optimum conditions for cross-linking of OVA by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Under the optimal conditions, structural changes in OVA were analyzed by circular dichroism, ultraviolet and fluorescence spectra. It was shown that CL-OVA became unordered and unfolded, and more tyrosine and tryptophan residues and hydrophobic groups were exposed onto the surface of molecules when compared to the native OVA. Enzyme-linked immunosorbent assay indicated that IgG and IgE binding abilities to OVA significantly reduced after cross-linking. All the findings demonstrated that enzymatic cross-linking in the presence of caffeic acid as a mediator may decrease the antigenicity and potential allergenicity, and the changes of the modified OVA were most likely a consequence of some changes or adjustment in the local conformation of OVA and the epitopes of OVA by cross-linking.

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1. Introduction

Hen's egg contains rich proteins that are beneficial to human bodies. However, it is also recognized as one of the most important food that causes allergic reactions, especially in infants and young children [1,2]. Thus far, allergic patients should absolutely avoid ingesting offending food, which is the only direct and dependable way to prevent egg allergy [3]. However, avoiding egg dishes or foods is extremely difficult because of the food diversity and the complexity of components in food products. By contrast, if eggs and egg ingredients products were removed from the diets of egg allergy sufferers, their nutritional balance and dietary pattern are bound to break [4]. Nevertheless, 50%–85% of egg allergic children could tolerate baked or heated eggs, which might be linked to the types of food that are accepted by patients with allergies to hen eggs [5].

Ovalbumin (OVA) is described as one of the major allergens in egg white, and it is also the most abundant protein in egg white, accounting for approximately 54% of the total protein [6]. Reasonably, reducing the allergenicity of OVA is of vital significance in the treatment of egg allergy. Moreover, OVA-induced allergy is considered mainly an IgE-mediated type I hypersensitivity [7], and the IgE binding epitopes including AA38–49, AA95–102, AA191–200, AA243–24, and AA251–260 presented on OVA were identified in sera from egg allergy patients [8] (Table 1).

It is well known that food processing can induce structural changes, which can influence the allergenicity by disrupting conformational or linear epitopes [9,10]. Stănciuc et al. [11] found OVA at alkaline pH value retained low levels of residual allergenic components heating at temperatures higher than 80 °C. Lechevalier et al. [12] tried to reduce the antigenicity of egg white by dry heat treatment. Compared with physically and chemically processed food proteins, as a new method of nonthermal processing technique in food industry, the improvements and modifications of proteins catalyzed by enzymes were widely studied, resulting in attracting

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Table 1
Information of egg allergic patients' sera.

Serum	Gender	Age (year)	Symptom	Specific IgE to egg white (IU mL^{-1})
1	Male	5	urticaria	>200
2	Male	2	asthma	>200
3	Male	1	Bronchial asthma	>100
4	Male	6	eczema	>200
5	Female	2	Bronchial asthma	>200
6	Male	2	eczema	>200
7	Male	1	Mycoplasma infection	>200
8	Female	30	Chronic urticaria	>200

more attention [13]. Actually, some documents are responsible for the enzymatic cross-linking of food proteins that can improve food texture, alter functionality, and influence digestibility and allergenicity [14–16]. Of the enzymes used for cross-linking proteins, polyphenol oxidases (PPOs), as one of the newly used enzymes, were caused wide concern [17,18].

Actually, PPOs are a group of copper-containing enzymes with complex composition, which summarize tyrosinases (EC 1.14.18.1), catechol oxidases (EC 1.10.3.1) and laccases (EC 1.10.3.2) [19,20]. They are oxidoreductases that are widespread in plants, fungus, and prokaryotic and eukaryotic organisms [21]. PPO can catalyze the hydroxylation of monophenol to dihydric diphenols and the subsequent oxidation of these dihydric diphenols to the respective o-quinones [22]. Regarding to proteins, PPO can catalyze the tyrosine (Tyr) residues to o-quinones. These quinones react spontaneously mainly via 1,4-additions with the $-NH_2$ or $-SH$ group of Tyr, lysine (Lys), or cysteine (Cys) residues to form intra- or intermolecular covalent cross-link [23]. Moreover, cross-linking between proteins with strongly defined structure or without Tyr residues can be induced by the small-molecule phenolic compounds as cross-linked mediators, such as caffeic acid. Caffeic acid can be oxidized by PPO into an o-benzoquinone, which reacts spontaneously with the $-NH_2$ or $-SH$ group of proteins to generate a caffeic acid-containing protein–protein cross-link [24,25].

Interestingly, the usage of PPO was anticipated as a novel approach to produce hypoallergenic foods [26]. Tantoush et al. [16] investigated the cross-linking potential of laccase on β-lactoglobulin (BLG) in the presence of sour cherry phenolics as a source of phenolic mediators, and the allergenicity of the cross-linked proteins was shown to be decreased in all nine cow's milk-allergic patients. Chung et al. [25] found that PPO treatment led to cross-linking of peanut extracts, resulting in the reduction of the allergenic properties of peanut allergens assessed by testing IgE binding ability. Recently, Wu et al. [27] also demonstrated that the modification of Ara h 2 molecules by PPO could decrease its potential allergenicity. However, information on egg allergens cross-linking catalyzed by PPO is not available yet. Accordingly, this work aimed to study whether OVA could be cross-linked catalyzed by PPO, followed by defining the effect of cross-linking on the potential allergenicity and structure changes of OVA.

2. Materials and methods

2.1. Materials

OVA with a purity of more than 98% was prepared by anion exchange chromatography on a DEAE-Sepharose Fast Flow column (16 mm × 250 mm, Amersham Biosciences, Freiburg, Germany) following the method of Ma et al. [28]. The protein solution was dialyzed against water at pH 7.0 for 48 h using 10 kDa molecular weight cutoff membranes, and then was lyophilized. The lyophilized OVA powder was stored at −20 °C. PPO powders from mushroom (*Agaricus bisporus*) were lab-made [18]. All other chemicals were purchased from the SigmaAldrich Corporation (St Louis, MO, USA).

2.2. Rabbit antibodies

Anti-OVA polyclonal antibodies IgG used for enzyme-linked immunosorbent assay (ELISA) were obtained in adult New Zealand rabbits according to the method of Ma et al. [29]. The sera were frozen at −80 °C until use.

2.3. Patients' sera

A pooled serum sample of eight subjects was used in this study. Subjects with a history of severe systemic reactions to eggs were provided by the First Affiliated Hospital of Guangxi Medical University, China, and were selected with informed consent. Egg allergy was diagnosed based on physical examination and objective clinical manifestations observed after egg ingestion. These sera were frozen at −80 °C until use.

2.4. Enzyme assay

Enzyme activity of PPO solution was measured using the method of Coseteng and Lee [30] with certain modifications. The sample contained 2.95 mL of 40 mM catechol solution in PBS (50 mM, pH 7.0) and 0.05 mL of PPO solution. Since the enzyme extract was added, the changes in absorbance at 420 nm were recorded every 30 s up to 3 min using a TU-1901 UV–vis spectrophotometer (Purkinje General Instrument Limited Liability Company, China). Each sample was detected in triplicate unless mentioned otherwise. The blank sample contained 2.95 mL of substrate solution and 0.05 mL of PBS. One unit of enzyme activity was defined as the increase in absorbance of 0.001 per min and per mL of the crude enzyme extract [31].

2.5. Experimental design for cross-linking conditions upon single factor

Ovalbumin (1.0 mg mL^{-1}) catalyzed by PPO of different enzyme activity (200, 400, 600, 800, 1000, and 1200 U mL^{-1}) in the presence of various caffeic acid concentrations (0, 0.1, 0.2, 0.5, 1, 2, 5, and 10 mM), was cross-linked at various temperatures (20, 30, 40, 50, 60, 70, and 80 °C), pH (4.0, 5.0, 6.0, 7.0, 9.0, and 10.0), ionic strengths (0.1, 10, 50, 100, and 200 mM, phosphate buffer solution, PBS), and times (1, 2, 4, 8, 12, and 24 h), respectively. When one factor was evaluated, the other factors were kept constant. The mixture were incubated in a constant temperature shaker. After the reaction, the cross-linked products were frozen and stored at −20 °C until use.

2.6. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE gels (10%) were prepared to assess the extent of OVA cross-linking caused by PPO. A PhastSystem Electrophoresis equip-

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