



Evaluation of the structure-activity relationship between allergenicity and spatial conformation of ovalbumin treated by pulsed electric field

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ABSTRACT

The aim of the present study was to investigate the effect of pulsed electric field (PEF) treatment on ovalbumin (OVA) induced allergens and reveal potential allergy regulatory mechanisms. At 10 kV/cm, OVA-induced allergic symptoms were significantly reduced, and the capacity of OVA to bind with specific IgG1 and IgE was reduced by 10.32% and 3.61%, respectively. Furthermore, the degranulation of RBL-2H3 cells and allergen activity were also reduced by 4.63% and 22.15%, respectively. Interestingly, the α -helix content was reduced by 5.81% and the fluorescence intensity was increased by 6.90% with PEF treatment. At 10 kV/cm, water contact angle and surface hydrophobicity increased by 8.40% and 0.18%, respectively, indicating that PEF treatment increased the exposure of hydrophobic amino acid residues. PEF treatment alters the hydrogen bonding and hydrophobic interactions in the protein, which masks the binding sites of sensitized epitopes, and consequently reduces allergies.

1. Introduction

Eggs are one of the main sources of dietary protein, and an abundantly utilized raw material in the food industry (Wen, Tu, Hu, & Wang, 2022). However, egg allergies have become the most common food allergy, surpassing children's allergies to milk (Zhang et al., 2020b); and children affecting up to 9% of children worldwide (Gazme, Rezaei, & Udenigwe, 2022). Egg allergens are concentrated predominantly in egg whites, and include ovomucoid, ovalbumin (OVA), ovotransferrin and lysozyme, among which, OVA is the most abundant allergen in egg white (Gazme, Rezaei, & Udenigwe, 2022; Liu et al., 2019). OVA is a spherical protein with a molecular weight of 45 kDa, comprising 385 amino acids, 4 thiol groups, of which there is an intramolecular disulfide bond in Cys73-Cys120 to form the stable "S" structure (Liao et al., 2018; Tu, Zhong, & Wang, 2017). The most frequent common amino acid sequences reported for IgE binding in OVA are 326–332 (AVHAAHA), 375–384 (AVLFGRGCVS) and 323–332 (ISQAVHAAHA) (Gazme, Rezaei, & Udenigwe, 2022). Although avoiding contact with foods containing egg components eliminates egg-related allergic reactions, this may lead to malnutrition in patients with allergies to eggs as well as other foods. Therefore, it is a great social significance to seek safe and fast methods to decrease the immunogenic properties of egg proteins with the aim of

reducing allergenicity. Recent, approaches applied to reduce allergy have entailed the modification of linear epitopes or spatial allergen conformations via glycation (Rupa & Mine, 2019), heat treatment (Liao et al., 2018), combination with other substances (Liu et al., 2019; Zhang et al., 2020a) and enzymatic hydrolysis (Liu et al., 2018b). The mouse model is an effective technical approach for studying allergy of various allergens (Liu et al., 2019). Evaluating the IgG and IgE binding ability of protein is another common method for determining allergy. In addition, IgE linear epitopes of OVA are present in nearly the entire amino acid sequence (Mine, & Yang, 2007; Zhang et al., 2020a). Finding new methods and technologies for the modification of linear epitopes or spatial conformations of proteins to reduce allergenicity has become a significant research topic in recent years.

New technologies for reducing the allergy of allergenic proteins through denaturation and interaction have been rapidly developed. Long et al. (2016) found that high pressure and thermal treatment significantly reduced the binding level of the peanut allergen Ara h 2 to specific IgE, and the level of histamine in the serum of BALB/c female mice. Yang et al. (2017) observed that high pressure and thermal treatment also reduced allergenicity to allergens in walnuts. Furthermore, physical processing combined with protease treatment can also reduce allergenicity, while high pressure-assisted crosslinking

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transglutaminase reduced the allergenicity of egg white protein (Ma et al., 2015), and high pressure and pepsin treatment have been found to reduce the allergenicity of whey protein (Lozano-Ojalvo et al., 2017). Because traditional thermal treatment reduces the nutritional value and quality of foods, the reduction of allergic components in food through nonthermal treatment has attracted increasing attention. Meinschmidt et al. (2016) established that pulsed ultraviolet light, remote cold atmospheric pressure plasma, and gamma-irradiation all reduced allergic reaction of soy protein isolates; direct cold atmospheric pressure plasma treatment provided the best results, and the immune response was almost completely suppressed. Zhou et al. (2016) observed that the allergenicity of ginkgo seed protein was reduced by 95% under a high hydrostatic pressure of 500 MPa, while it was nearly entirely absent at 600 and 700 MPa. Changes in allergenicity are closely related to the allergen structure. Zhang et al. (2017) noted that the tropomyosin particle size, and α -helix and total sulphydryl group contents were significantly reduced following ultrasonic treatment at 800 W for 15 min. Moreover, tropomyosin allergenicity was also significantly decreased, which indicated that high-intensity ultrasound treatment reduced the allergenicity by reducing structural stability. In their studies on shrimp tropomyosin, Dong et al. (2020) used tropomyosin in shrimp as the research object, and Wang et al. (2021) found that the allergenicity was significantly reduced when the spatial protein conformation was modified; this finding was also observed by Wang et al. (2021) during their research into kiwifruit Act d 2 proteins. However, Lavilla et al. (2016) noticed that the allergy potential of the principal allergen in peaches (Pru p 3) increased after high pressure treatment, indicating that processing methods are an important factor in the management of allergen risks in the food industry.

In recent years, numerous studies on the application of non-thermal processing technologies for inhibiting the allergenicity of allergenic proteins have been published, but reports regarding the effects of PEF treatment on allergenic proteins are scarce. Therefore, the purpose of study was to evaluate the effects of PEF treatment on the allergen and spatial conformation of OVA using indirect sandwich ELISA and traditional spectroscopy techniques, and by determining cell degranulation levels, as well as to explore the PEF allergy inhibition mechanism. The findings of this study will provide technical support for the production of hypoallergenic or non-allergenic egg products, and promote the application of emerging food processing technologies in the food industry.

2. Materials and methods

2.1. Reagents and materials

OVA (A5503, molecular weight: 45.0 kDa, purity $\geq 99.40\%$) was obtained from Sigma-Aldrich (St. Louis, MO). Tyrode's solution was obtained from Beijing Solarbio Technology Co., Ltd. (Beijing, China). RBL-2H3 cells were purchased from the Cell Resource Center of the Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). Goat anti-mouse IgG1 (HRP) pre-adsorbed secondary antibody (ab98693), Rat monoclonal antibody [23G3] anti-mouse IgE epsilon chain (ab99585), Streptavidin (HRP) (ab7403) and TMB ELISA substrate (ab171523) were purchased from Abcam Trading Co., Ltd. (Shanghai, China). Fetal bovine serum was purchased from PAN-Biotech GmbH (Aidenbach, Germany). MEM medium and Imject™ Alum Adjuvant was obtained from Thermo Fisher Scientific (Shanghai, China). All other chemical reagents used in the experiment were of analytical grade.

2.2. OVA-induced mouse food allergy model

Four-week-old female BALB/c mice were obtained from Changsheng Biotechnology Co., Ltd. (Dalian, China). All animal protocols were approved by the Animal Ethics Committee of Dalian Polytechnic University (Dalian, China, NO. SYXK2017-0005). Mice were placed in an environment of $25 \pm 1^\circ\text{C}$ and a dark/light cycle of 12 h, during which

they were allowed to eat food and drink water ad libitum.

After five days of adaptation, the sixty mice were divided into control and OVA-induced allergy groups ($n = 30$ mice in each group). On days 0, 3, 6, 9 and 12, intraperitoneal (i.p.) injections, comprising 100 μg of OVA dissolved in 100 μL PBS and 100 μL Imject Alum were administered to each mouse in the OVA-induced allergy group. The control group was injected with PBS instead of OVA. Subsequent to the i.p. injections, blood was collected from the orbital plexus on each of the five days to determine antibody activity. After the final blood collection, the mice were fasted for 24 h and then euthanized by dislocation of the cervical vertebra (Liu et al., 2019).

2.3. PEF treatment

A stock solution of OVA (5 mg/mL) was prepared in water. A self-designed pulsed electric field system DMC-200 was used to process the OVA solution. The PEF treatment conditions were as follows: flow rate, 3.2 mL/min; frequency, 2000 Hz; and electric field intensity, 10, 20, and 30 kV/cm. The sample size for each electric field intensity experiment was 6.0 mL (Zhang et al., 2021b).

2.4. Determination of OVA binding levels to total or specific IgG1 and IgE

The levels of total IgG1 and IgE in mouse serum were determined using an ELISA kit, according to the manufacturer's instructions. The binding abilities of specific IgG1 and IgE were determined employing a previously published protocol (Li et al., 2021; Rupa, & Mine, 2019). Briefly, 100 $\mu\text{g}/\text{mL}$ OVA solution dissolved in carbonic acid buffer (pH 9.6) was coated in a 96-well ELISA plate (Costar Corning Inc., Corning, NY) and kept overnight at 4°C . The plate was then washed twice with PBS/0.1% BSA-Tween 20 (PBST) washing solution and incubated for 60 min in PBS/1% BSA solution at 37°C . After washing three times with PBST, 100 μL of the allergy serum diluted with PBST (IgG1 dilution was 1:100; IgE dilution was 1:10) was added to each well, followed by incubation for 120 min at 37°C . After six washes with PBST, 100 μL of IgG1-HRP (1:3000, v: v) or IgE (1:2000, v: v) were added to each well, followed by incubation for 60 min at 37°C . Specific IgG1 antibody binding was measured after six washes; 100 μL TMB was added to each well and incubated for 15 min at 37°C , followed by the addition of 50 μL of 1 mol/L H_2SO_4 to terminate the color reaction and the absorbance was measured at 450 nm. Specific IgE antibody binding was measured after six washes; 100 μL of HRP (1:4000, v: v) was added each well, followed by incubation at 37°C for 60 min. After six washes, 100 μL of TMB was added to each well, followed by incubation at 37°C for 15 min. Next 50 μL of 1 mol/L H_2SO_4 was added to terminate the color reaction and absorbance was measured at 450 nm.

2.5. Determination of RBL-2H3 cell degranulation

RBL-2H3 cells (2×10^5) were added to 96-well plates and cultured for 24 h at 37°C . The plates were washed with PBS and the cells were cultured with allergy serum (100 μL , diluted to 1:10) for 24 h. The cells were washed three times with tyrode's solution and then treated with OVA samples of varying concentrations (0.01, 0.1, 1, 10 and 100 ng/mL) at 37°C for 45 min. The cells were placed in an ice box to stop the reaction, then 30 μL of supernatant and 50 μL of 4 - nitrophenyl Nacetyl - β - D - glucosaminide (1.3 mg/mL in 0.2 mol/L citric acid buffer solution) were added to the 96-well plate. After incubation at 37°C for 60 min, 200 μL of 0.2 mol/L sodium carbonate buffer was added the absorbance was measured at 405 nm. To determine the total release of β - hexosaminidase 1% Triton X-100 was used instead of the OVA samples, and for the spontaneous release, tyrode's solution was used instead of the OVA samples (Sun et al., 2014).

2.6. SDS-PAGE gel electrophoresis

Changes in the molecular weight of the OVA samples following PEF treatment were estimated using SDS-PAGE. The OVA stock solution was diluted to 1:4 (v/v) using a sample buffer (0.25 mol/L Tris-HCl, 10% SDS, 0.5% Bromophenol blue, 50% Glycerol, 5% β -mercaptoethanol). The samples were loaded using 10 μ L per lane, and 7.5 μ L of a molecular weight marker (14.4–116 kDa) (Thermo Fisher Scientific Co., Ltd. Shanghai, China) was also loaded. Then, using 5% stacking gel and 12% polyacrylamide separation gel, electrophoresis was conducted at 60 V for 30 min and 100 V for 120 min at 4°C, followed by dyeing for 120 min in 0.1% Coomassie Brilliant Blue R-250 (Sigma-Aldrich Chemical, St. Louis, MO, USA) staining solution and destaining in decolorizing solution for 24 h. Finally, the ChemiDoc™ touch imaging system (Bio-Rad Co., Ltd. California, USA) was utilized for image capture (Li et al., 2021).

2.7. Circular dichroism spectroscopy

The CD spectra of the OVA samples were obtained using a J-810CD spectrometer (Jasco Co., Japan) at $25 \pm 1^\circ\text{C}$ and a scan rate of 20 nm/min, within scan range of 190 to 250 nm, using quartz cuvettes with a width of 1.0 cm. The contents of four types of secondary structures (α -helix, β -sheet, β -turn, and random coil) were analyzed using the Cdpso software package (Zhang et al., 2021a). An average of three scans is reported.

2.8. Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectra of OVA the samples were acquired with a Fourier Transform Infrared Spectrometer (PerkinElmer, USA) at $25 \pm 1^\circ\text{C}$. Each sample and KBr were mixed in a ratio of 1:100 and then pressed into discs. The measurement conditions were as follows: scanning range was 400–4000 cm^{-1} , the resolution was 4 cm^{-1} , the number of scans was 32, and air was used for the background scan. The spectra were analyzed employing OMNIC 8 software (Thermo Nicolet Co., Madison, WI) and curve normalization was performed using OriginPro 8.5 software (Origin Lab Corporation, Northampton, MA, USA) to analyze OVA conformational changes (Lu et al., 2018).

2.9. Ultraviolet spectroscopy

According to the method of Zhang et al. (2020a) a UV/VIS Lambda 35 spectrophotometer (PerkinElmer, USA) was used to obtain the UV absorption spectra of OVA samples. The UV spectrum of OVA was measured in a quartz cuvette with a concentration of 0.5 mg/mL and an optical path length of 1.0 cm. The spectral scan was 200–500 nm at a scan speed of 480 nm/min.

2.10. Fluorescence spectroscopy

The effects of PEF treatment on the OVA fluorescence spectrum were evaluated according to the method detailed by Liao et al. (2018). The intrinsic fluorescence intensity of OVA was measured at an excitation wavelength of 280 nm and the emission spectrum was obtained in the range of 290–500 nm using a fluorescence spectrophotometer (F-2700, Hitachi, Tokyo, Japan).

2.11. Water contact angle measurements

The method described by Zhang, Zeng, Liu and Cai (2021) was used with slight modifications to determine the effect of PEF treatment on the water contact angle (WCA) of the OVA samples, employing the sessile drop method. WCA imaging was achieved using a BDA25S (Hamburg, Cruise, Germany) WCA meter equipped with image analysis software; 2.0 μ L deionized water droplets were deposited on the air side of the OVA sample membrane surface using a precision syringe. At least six

parallel experiments were performed for each sample.

2.12. Surface hydrophobicity measurements

To the method reported by Liao et al. (2018) was used to measure, the surface hydrophobicity (H_0) of OVA by a fluorescence spectrophotometry (F-2700, Hitachi, Tokyo, Japan) using 8-Anilino-1-naphthalenesulfonic acid (ANS) as a fluorescent probe. In short, the native OVA sample and PEF-treated OVA samples were diluted to 0.1, 0.2, 0.3, 0.4 and 0.5 mg/mL, and then 20 μ L of ANS (8 mmol/L in Tris-HCl buffer, pH 8.0) was injected to 4 mL of the OVA solution. After 15 min of reaction in the dark at 25°C , fluorescence intensity was measured at an excitation wavelength of 390 nm and emission wavelength of 470 nm. The H_0 value was derived from the slope of the fitted curve generated by plotting the fluorescence intensity as a function of protein concentration.

2.13. Statistical analysis

All images were plotted and modified using Origin Pro 8.5 software. All data were tested for significance with SPSS Statics 17 (SPSS Inc. USA), $P < 0.05$ indicated a significant difference.

3. Results and discussion

3.1. PEF-induced inhibition of OVA binding with specific IgG1 and IgE

The plots of total IgG1 and IgE mouse serum levels over time are shown in Fig. 1 (A–B). The control group levels did not increase significantly, while in the OVA group, they increased significantly from the 5th until the 15th day, and no significant difference in the levels was observed between the 15th and 20th day. Furthermore, the control and OVA-induced allergy groups contained significantly different levels from the 10th day, which indicated that the animal allergy model was successfully constructed. Therefore, the mice serum extracted on the 20th day was selected to determine the inhibitory effect of PEF treatment on the binding ability of OVA with specific IgG1 and IgE (Fig. 1C and D). Compared with the results at 0 kV/cm, the IgG1 binding capacity of OVA was reduced by 10.32%, 7.70% and 4.77%, when the electric field intensities were 10, 20, and 30 kV/cm, respectively. In addition, the IgE binding capacity of OVA was decreased by 3.61% and 2.78% under low electric field intensities (10 and 20 kV/cm, respectively), while at a high electric field intensity of 30 kV/cm, it increased by 1.22%. Liu et al. (2019) reported that the OVA capacity for binding to specific IgE and IgG1 was slightly reduced by coumarin, but that for specific IgG2a increased slightly. Tong et al. (2012) observed that the ovotransferrin (OVT) ability to bind to specific IgG increased when the thermal treatment temperature was 55°C and 60°C , while it decreased when it was $\geq 80^\circ\text{C}$. Furthermore, the OVT binding capacity for specific IgG increased after thermal treatment for 5 min when the temperature was 70°C , while it showed a decreasing trend with increasing thermal treatment time. The above results indicated that the ability of protein to bind specific antibodies, such as IgE, IgG, IgG1 and IgG2a may exhibit varying trends after treatment. At 40 kV/cm, the binding ability of OVA with specific IgG1 decreased and the binding ability with specific IgE was slightly increased, indicating that PEF treatment had different effects on the binding ability of OVA with specific IgG1 and IgE.

3.2. Changes in RBL-2H3 cell degranulation in OVA following PEF treatment

To evaluate the anti-allergic effects of PEF treatment on OVA samples, RBL-2H3 cells were stimulated with the antigen and allergy serum, and released β -hexosaminidase was quantified. The β -hexosaminidase levels differed significantly depending on the electric field intensity, and RBL-2H3 cell degranulation increased in a dose-dependent manner (Fig. 2A). The optimal concentration at which β -hexosaminidase release

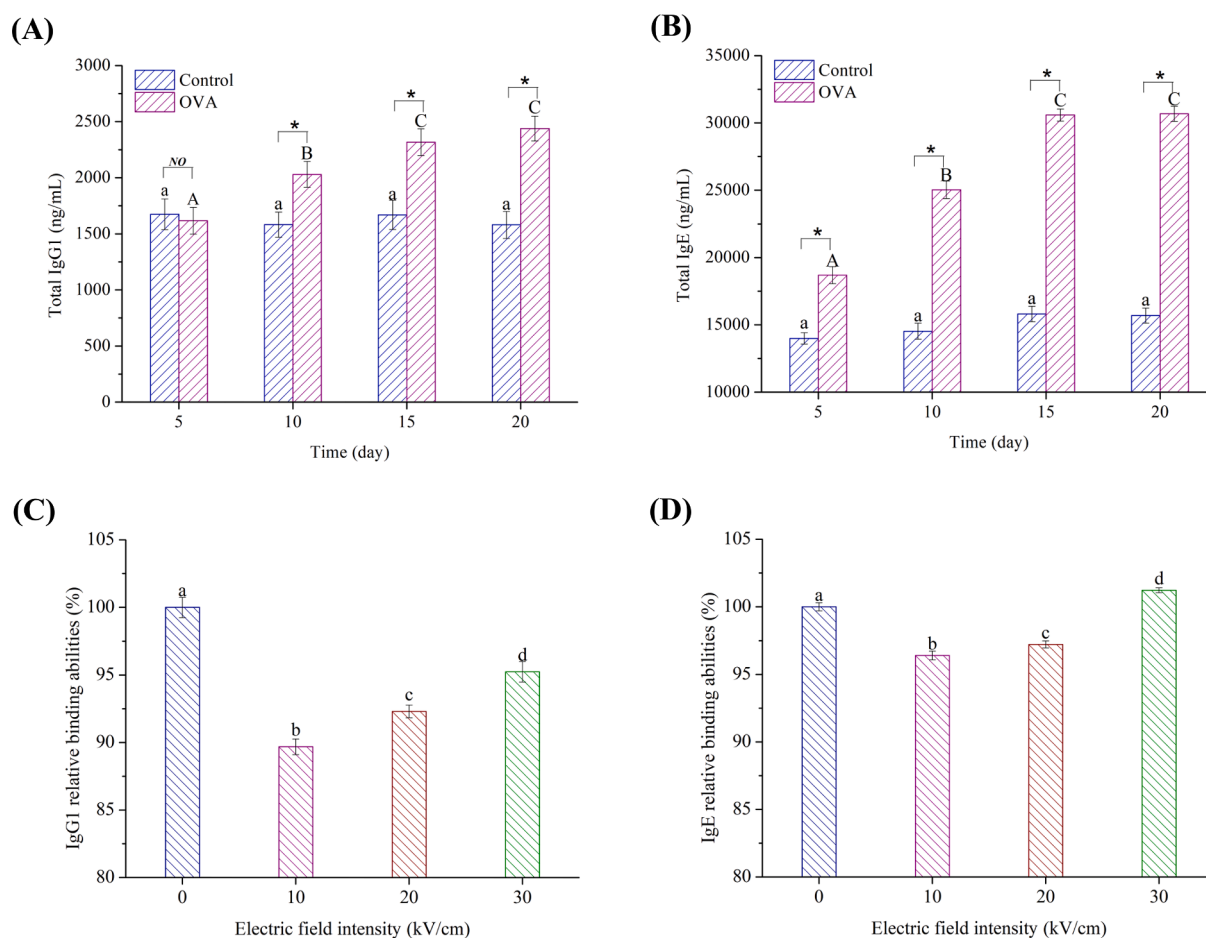


Fig. 1. The levels of total IgG1 (A) and IgE (B) in mouse serum at different times. PEF treatment inhibits the binding ability of OVA with specific IgG1 (C) and IgE (D). Data are expressed as means $\pm$ standard deviation ($n = 3$). Different letters indicate statistically significant differences ($P < 0.05$).

was lowest was 10 ng/mL. Furthermore, we found that PEF treatment inhibited β -hexosaminidase release. Briefly, the inhibitory effect on β -hexosaminidase release was most significant at 10 kV/cm, with levels decreasing from 37.76% at 0 kV/cm to 33.13% at 10 kV/cm. Furthermore, we also noticed that β -hexosaminidase release tends to increase with increasing electric field intensity. At the same time, it was observed that PEF treatment also had a significant effect on the EC₅₀ of OVA (Fig. 2B). The EC₅₀ decreased with increasing electric field intensity, indicating that the inhibition of OVA allergic activity decreased with increasing electric field intensity.

Overall, optimal allergy inhibition was achieved at 10 kV/cm, as determined from the capacity of OVA to bind to specific antibodies IgG1 and IgE, and the levels of released β -hexosaminidase. PEF treatment likely inhibits OVA-induced allergy by inducing changes in the OVA secondary structure or microenvironment of amino acid residues, thereby masking its immunogenic epitope (Yang et al., 2020). Wei et al. (2018) suggested that the tertiary structure of OVA was more susceptible to PEF treatment than the secondary structure. When the treatment time was 4320 μ s of tryptophan and tyrosine exposure increased by 30.00% and 43.75%, respectively. Zhao and Yang (2008) noticed that after PEF treatment, the secondary structure was partially unfolded through lysozyme activity and the α -helix content was reduced. Moreover, the tertiary structure was also significantly unfolded, leaving tyrosine residues embedded in the protein molecule, and tryptophan residues more exposed to the microenvironment. Therefore, it was necessary to explore the effect of PEF treatment on the spatial structure of OVA samples, and to reveal the mechanism by which PEF treatment inhibits the immunogenic binding epitope.

3.3. Changes in the molecular weight of OVA under PEF treatment

SDS-PAGE is a crucial tool for evaluating changes in protein primary structures (Wang, Wang, Vanga, & Raghavan, 2021). The influences of PEF treatment on the SDS-PAGE of OVA samples are shown in Fig. 3. The molecular weight at 0 kV/cm was 45.0 kDa, which was consistent with the previously reported value (Ma et al., 2015). In addition, the mobilities of OVA sample bands did not differ significantly, which indicated that PEF treatment marginally altered the molecular weight of the protein. Meng et al. (2018) also noticed that the molecular weight of the red bean seed ferritin subunit did not change with PEF treatment, while it maintained the classic cage structure. Furthermore, we have previously reported that the molecular weight of ovomucoid did not change significantly after PEF treatment (Li et al., 2021). The above results indicated that PEF treatment had no significant effect on the primary structure of OVA, which may be because the instantaneous high voltage effect of the electric field has no significant on the covalent inter- or intramolecular bonding in the protein molecules, hence, new covalent bonds were not formed to cause protein molecules to aggregate. Thus, the effect of PEF treatment on the secondary and tertiary structure of OVA was further investigated to reveal the mechanism by which PEF treatment inhibits OVA allergy.

3.4. Changes in the OVA secondary structure under PEF treatment

CD spectroscopy is an important technical tool for investigating changes in the secondary structure of protein samples (Chang et al., 2020). The effect of PEF treatment on the CD spectrum of the OVA is

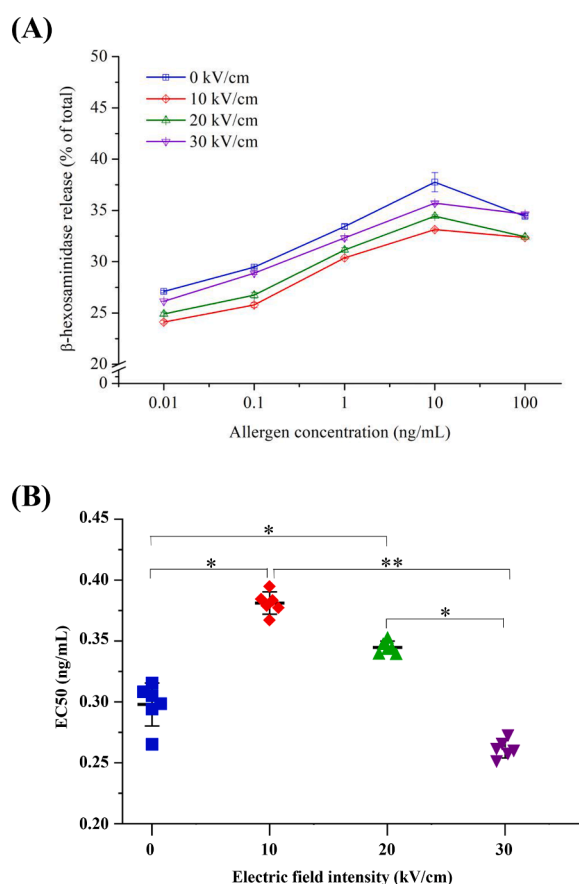


Fig. 2. (A) Detection results of β -hexosaminidase release from sensitized RBL-2H3 cells induced in OVA by PEF treatment. (B) Detection results of EC50 values of sensitized RBL-2H3 cells induced in OVA by PEF treatment. Data are expressed as means $\pm$ standard deviation ($n = 3$). Different letters indicate statistically significant differences ($P < 0.05$).

shown in Fig. 4A. The CD spectra of the OVA samples contained one positive band at 193 nm and one negative band at 210 nm, which were characteristic of the α -helix structure in proteins (Liu, Ying, Cai, & Le, 2017). The one negative band at 222 nm was characteristic of the β -sheet structure. In addition, the intensity of the CD spectrum changed after PEF treatment, indicating that the secondary structure content in the OVA sample had changed. The α -helix, β -sheet, β -turn and random coil contents were quantified according to the CD spectrum in Fig. 4A, and are shown in Fig. 4B. The 0 kV/cm group contained 46.47% of α -helix, 14.53% of β -sheet, 12.33% of β -turn and 26.67% of random coil. Comparatively, the α -helix and β -sheet contents decreased significantly at 10 and 20 kV/cm, indicating breakage of intramolecular hydrogen bonds and partial unfolding of the α -helix by PEF treatment (Dong et al., 2021; Zhang et al., 2020a). Furthermore, the α -helix and β -sheet contents increased with the increasing electric field intensity, which was consistent with the finding that the inhibitory effect of PEF treatment on OVA allergy gradually decreased with increasing of electric field intensity.

FTIR spectral analysis was used to further study the conformational changes of OVA with PEF treatment. The PEF-induced changes in the FTIR spectrum of the OVA samples are shown in Fig. 4C. A broad peak was observed at 3200–3600 cm^{-1} , which was attributed to overlapped O—H and N—H stretching vibrations (Liu, Ying, Cai, & Le, 2017). The peak intensity increased after PEF treatment, indicating that PEF treatment changed the state of the amino acid residues in the protein. Characteristic amide I and amide II bands of the 0 kV/cm group were located at 1646.78 cm^{-1} and 1544.05 cm^{-1} , respectively; at 10 kV/cm, these peaks were band blue shifted to 1647.10 cm^{-1} and 1544.38 cm^{-1} ,

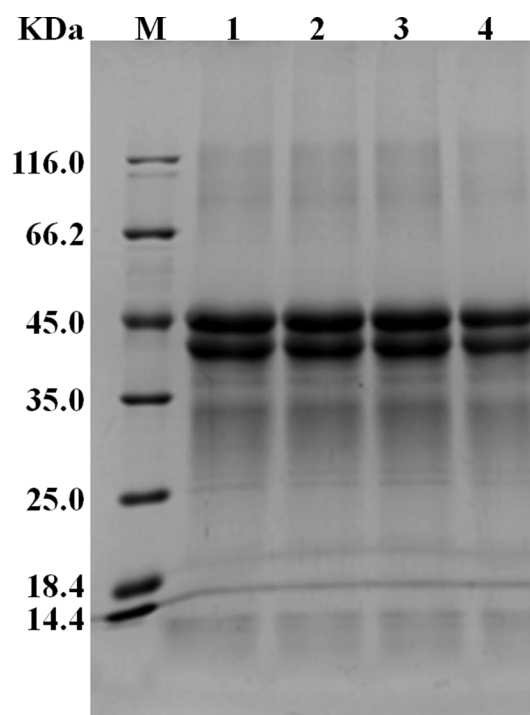


Fig. 3. SDS-PAGE patterns of OVA. Lane M: mean marker; lane 1: mean native OVA; lane 2: mean OVA processed at 10 kV/cm; lane 3: mean OVA processed at 20 kV/cm; lane 4: mean OVA processed at 30 kV/cm.

respectively. Moreover, with increasing electric field intensity, the amide I and the amide II bands gradually red shifted. This suggested that PEF treatment altered the C=C stretching vibration and C—N in-plane bending vibration of the OVA samples (Liu, Ying, Cai, & Le, 2017; Sun et al., 2020), which mutually corroborated the changes in the secondary structure deduced from the CD result. In addition, the intensities of amide I and amide II bands increased, which was caused by the increased exposure of hydrophobic groups and the breakage of hydrogen bonds induced by PEF treatment (Sheng et al., 2020). The CD and FTIR spectroscopy results both indicated that PEF treatment changed the secondary structure of OVA, which resulted in partial uncoiling of the α -helix and exposure of hydrophobic groups. The secondary structure changes altered the allergy site; therefore, PEF treatment inhibited the allergy of OVA to a certain extent.

3.5. Changes in the OVA tertiary structure with PEF treatment

UV and fluorescence spectroscopy are commonly used to characterize the tertiary structure of proteins. Aromatic amino acids such as tryptophan, tyrosine, and phenylalanine are present in OVA (Zhang et al., 2020a). These amino acids can behave as chromophores and have a maximum absorption peak near 280 nm in the UV absorption spectrum, which can be used for protein structure analysis (Hu et al., 2021). As shown in Fig. 5A, the maximum OVA absorption peak was 280 nm, attributed to the conjugated double vibration of tryptophan and tyrosine. The UV absorption intensities of the OVA samples first increased and then decreased with increasing electric field intensity, following the order of 10 kV/cm > 0 kV/cm > 20 kV/cm > 30 kV/cm. The changes in the UV absorption intensity indicated that PEF treatment resulted in cleavage or formation of hydrogen bonds and hydrophobic interactions, leading to changes in the exposure of tyrosine and tryptophan (Nnyigide, Lee, & Hyun, 2018). These changes cause unfolding or contraction of the protein structure (Chang et al., 2020) which alter the immunogenic epitope of OVA and consequently inhibit OVA allergic reaction.

Intrinsic protein fluorescence is usually produced by tryptophan,

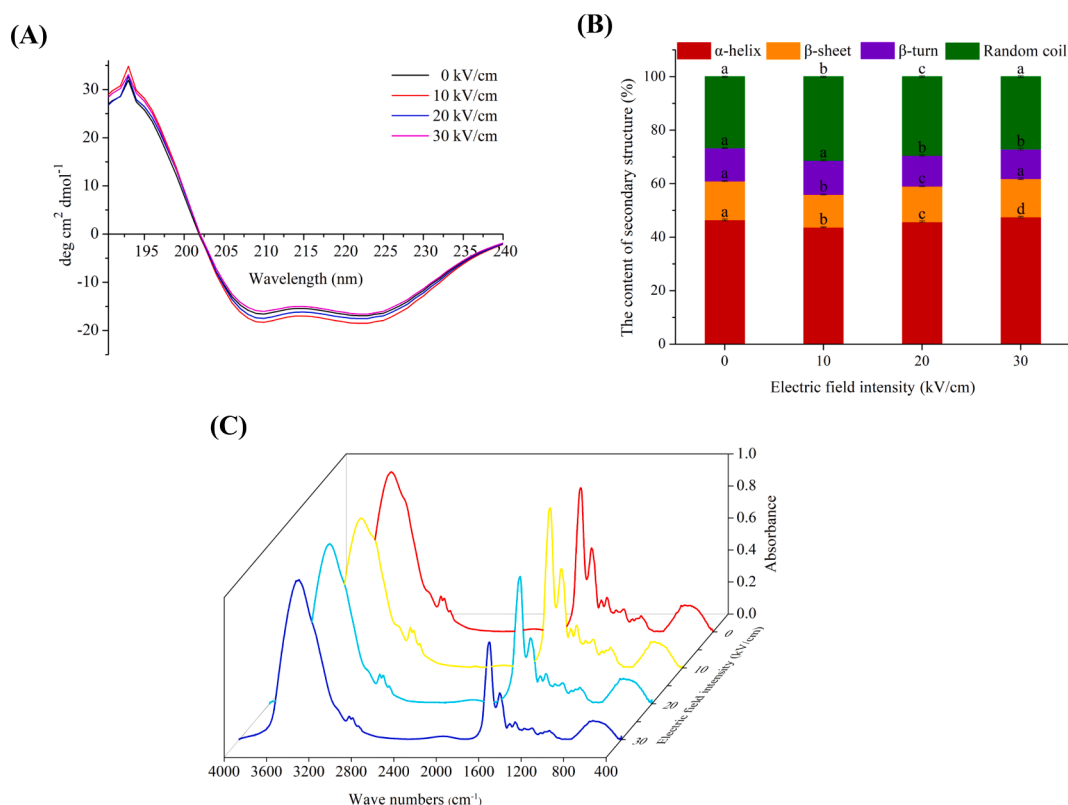


Fig. 4. Influence of PEF treatment on the secondary structure of the OVA. (A) CD spectrum, (B) secondary structure content, (C) FTIR spectrum. The same index used different letters to indicate the significant difference ($P < 0.05$).

phenylalanine, and tyrosine, among which, tryptophan is the main amino acid responsible for intrinsic fluorescence (Liao et al., 2018). The fluorescence produced by tryptophan is readily quenched by the environment; thus, the effect of PEF treatment on the intrinsic fluorescence of OVA was studied (Fig. 5B). The fluorescence emission maximum was red-shifted from 340.5 nm at 0 kV/cm to 344.5, 344.0, and 343.5 nm at 10, 20, and 30 kV/cm, respectively. This indicated that PEF treatment changed the polarity of the microenvironment around the tyrosine in OVA (Yang et al., 2020). In addition, the fluorescence intensity increased at 10 and 20 kV/cm, indicating that the chromophore became buried in the molecule to a greater extent during the partial unfolding caused by PEF treatment. The result was consistent with the finding that the fluorescence intensity of horse ferritin increased under a low electric field intensity (Zhang et al., 2021a). The fluorescence intensity decreased at 30 kV/cm, indicating that when the OVA was tightly packed, the chromophore was exposed to the solvent and interacted with the quencher to reduce the fluorescence intensity (Hu et al., 2019). Both the UV and the fluorescence spectra indicated that the changes in the tertiary structure of OVA induced by PEF treatment had masked the allergic binding site, thereby inhibiting allergy.

3.6. Changes in the WCA and H_0 in OVA under PEF treatment

Hydrophobicity is a non-covalent intermolecular force involved in immune response and plays a key role in assisting in the recognition of specific paratope epitopes between antibodies and antigens (Liu et al., 2018a). The FTIR, UV and fluorescence spectra revealed that PEF treatment increased the exposure of hydrophobic groups in OVA; therefore, we further evaluated the effect of PEF treatment on the protein hydrophobic groups by determining the WCA and H_0 . The hydrophobic and hydrophilic amino acid residue content on the OVA molecular chain is roughly equal, and most of the hydrophobic amino acid residues are buried inside the molecule (Zhang, Zeng, Liu, & Cai,

2021). The effect of PEF treatment on the WCA of the OVA samples are shown in Fig. 6A. The WCA of the OVA group treated at 0 kV/cm was 50.35°, which indicated that most of the hydrophobic amino acid residues were indeed embedded in the molecule, and it also verified that OVA was a hydrophilic protein. Under low electric field intensities (10 and 20 kV/cm), the WCA of OVA increased, while under a high electric field intensity (30 kV/cm), the WCA decreased. The results corroborated the changes in the hydrophobic groups observed in the FTIR and UV spectra. Exposure of the hydrophobic groups was caused by partial unfolding of the α -helix and breakage of intramolecular hydrogen bonds (Dong et al., 2021). The α -helix content increased and the structure was packed tighter under a high electric field intensity, therefore the hydrophobic groups were better buried inside the molecule, and the WCA was significantly reduced at 30 kV/cm compared with that of the 0 kV/cm group.

H_0 is another important index affecting hydrophobic interactions, and we measured it to further analyze the effect of PEF treatment on the hydrophobic groups in the OVA molecular chain (Fig. 6B). The H_0 increased under low electric field intensities (10 and 20 kV/cm), but it decreased under a high electric field intensity (30 kV/cm), which its changed trend consistent with that of WCA. These results were consistent with those of previous studies on ovomucoid where in PEF treatment was shown to have a significant effect on the H_0 of the protein (Li et al., 2021). In addition, ANS preferably binds to lysine and arginine, and as PEF treatment altered amino acids exposure (Yang et al., 2020), the binding between ANS and OVA was altered, resulting in an increase in H_0 . Both WCA and H_0 indicated that PEF treatment of OVA enhanced hydrophobic interaction under low electric field intensities, and diminished them under a high electric field intensity. As hydrophobic interaction play an important role in antibody and antigen recognition and binding, PEF inhibition of OVA allergic reaction at 10 and 20 kV/cm.

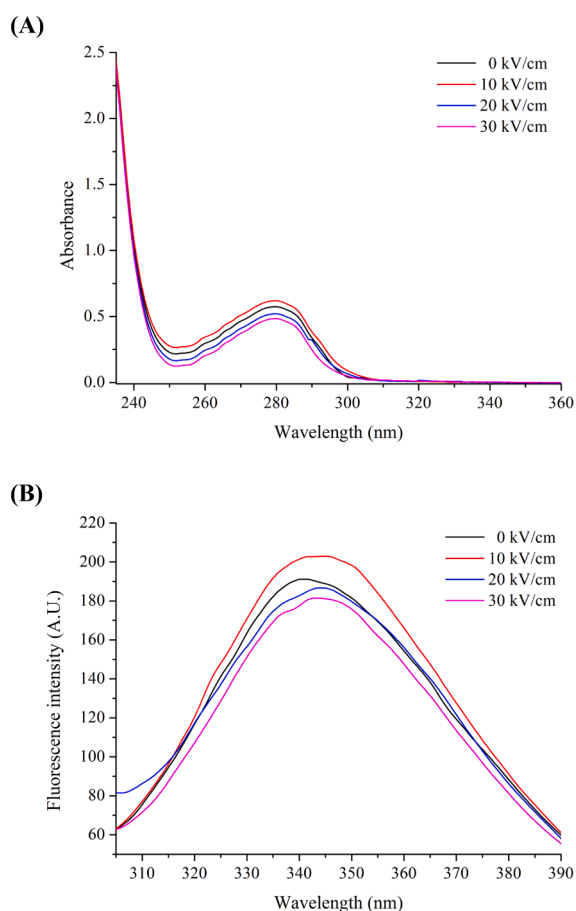


Fig. 5. Influence of PEF treatment on the tertiary structure of the OVA. (A) UV spectrum, (B) fluorescence spectrum.

4. Conclusions

In conclusion, this study evaluated the effects of PEF treatments at varying different electric field intensities on the allergenicity and spatial conformation of OVA samples. Compared with the ability of OVA to bind IgG1 and IgE at 0 kV/cm, PEF treatment inhibited this capacity, and was highest at 10 kV/cm. In addition, the maximum release rate of β -hexosaminidase was also inhibited by PEF treatment, and the inhibition rate was the highest at 10 kV/cm and 10 ng/mL, with allergic reaction being 77.85% of that at 0 kV/cm. The spatial conformation was changed after PEF treatment, and any treatment that changes the conformational of the protein may interfere with the ability of antibodies recognition. Therefore, allergic reaction was decreased in OVA, primarily due to the partial uncoiling of the secondary structure and the increased exposure of the hydrophobic region of the tertiary structure. This study offers valuable insights into the mechanism of PEF-induced changes in allergenicity and structure of OVA and provides a theoretical basis for the development of hypoallergenic or non-allergenic egg products.

CRediT authorship contribution statement

Yinli Li: Data curation, Formal analysis, Methodology, Visualization, Writing – original draft. **Shuyu Zhang:** Methodology, Visualization. **Jie Ding:** Data curation, Methodology. **Limin Zhong:** Investigation, Software. **Na Sun:** Conceptualization, Formal analysis, Funding acquisition. **Songyi Lin:** Conceptualization, Validation, Supervision, Supervision, Project administration, Writing – review & editing.

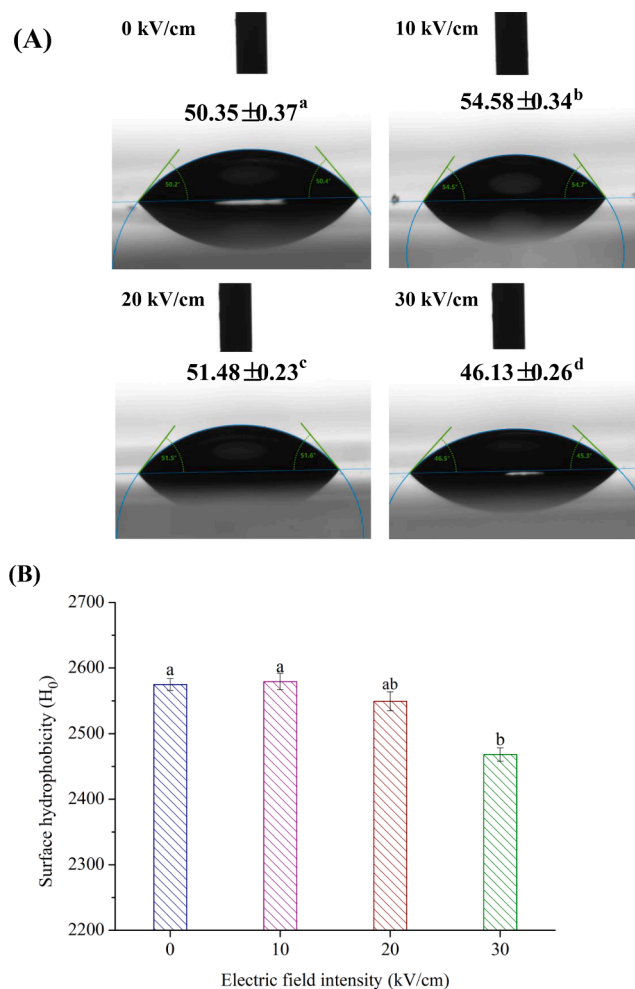


Fig. 6. Influence of PEF treatment on the tertiary structure of the OVA. (A) water contact angle, (B) surface hydrophobicity. The same index uses different letters to indicate the significant difference ($P < 0.05$).

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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