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Establishment of a Highly Sensitive Sandwich Enzyme-Linked Immunosorbent Assay Specific for Ovomucoid from Hen's Egg White

YONGMING LI, CHAOJUN SONG, KUI ZHANG, MEILIANG WANG, KUN YANG, ANGANG YANG, AND BOQUAN JIN*

Department of Immunology, The Fourth Military Medical University, Xi'an 710032, China

A highly sensitive sandwich enzyme-linked immunosorbent assay (ELISA) kit was established for quantifying ovomucoid from hen's egg white, which has been considered as one of the major allergen in egg white. The detection limit reached 0.041 ng/mL, and linearity ranged from 0.1 to 6.25 ng/mL. Intra- and interassay coefficient variations were all lower than 5% at three concentrations (0.5, 2.5, and 5 ng/mL). No cross-reactivity was observed with bovine serum, horse serum, goat serum, human serum, duck egg white, goose egg white, quail egg white, and pigeon egg white, but a low level of cross-reactivity was found with chicken serum. The ELISA kit was established on the basis of two monoclonal antibodies (mAbs) recognizing different epitopes of ovomucoid. However, these mAbs were generated using commercially purified ovalbumin as immunogen. Studies on the relative allergenicity and antigenicity of egg white protein have been performed by many researchers, but there were controversial opinions reported previously because of the impurity of each egg white protein used in various studies. In the present work we measured the degree of ovomucoid contamination in commercially purified ovalbumin sample, and the value was about 11%. We also determined the ovomucoid residue in influenza vaccine samples for the first time. These data showed that the ELISA kit we established could serve as an effective method for precisely quantifying concentrations of ovomucoid in the egg industry and as a useful tool for the research of allergenicity and antigenicity of hen's egg proteins.

KEYWORDS: Egg white; ovomucoid; allergy; monoclonal antibody; ELISA

INTRODUCTION

Hen's egg is the most frequent cause of food allergy in children, and approximately two-thirds of children diagnosed with a food allergy are reactive to egg white (*1, 2*). Egg white contains proteins with higher allergenic potential than egg yolk. Among different proteins in egg white, 4 strong allergens have been identified; these include ovalbumin, ovomucoid, ovotransferrin, and lysozyme (*3, 4*). However, ovomucoid is considered one of the major allergen in egg white despite the existence of controversial reports; the contamination of ovomucoid in commercially purified ovalbumin had led to an overestimation of the dominance of ovalbumin as the major egg allergen (*5–9*).

Ovomucoid is a glycoprotein (22–29% carbonhydrate) with a molecular weight of 28 kDa that contains 186 amino acid residues and comprises 11% of the total egg white protein (*3, 6*). In the previous studies, several methods were used to quantitatively analyze the amount of ovomucoid, including SDS-PAGE, Western blot, analysis of high-performance liquid chromatography (HPLC) profile, and sandwich enzyme-linked immunosorbent assay (ELISA) (*3, 8, 10–12*), but the sensitivity and/or specificity were still limited.

When we attempted to generate mouse monoclonal antibodies (mAbs) to hen's egg ovalbumin using commercially purified ovalbumin as an immunogen, two of the nine clones generated were found to be specific for ovomucoid because of the contamination of ovomucoid in commercially purified ovalbumin. On the basis of the two mAb clones to ovomucoid, we established a highly sensitive sandwich ELISA specific for hen's egg ovomucoid. The high sensitivity and specificity made the precise measurement of hen's egg ovomucoid possible. As a potent detection marker of egg white allergen, the amount of ovomucoid residue was measured in influenza vaccine samples. We also determined the degree of ovomucoid contamination in commercially purified ovalbumin samples and discussed the allergenicity and antigenicity of hen's egg ovomucoid.

MATERIALS AND METHODS

Reagents. Goat antimouse IgG and BCA protein assay kit were purchased from Pierce (Rockford, Illinois), SBA Clonotyping System was from SouthernBiotech (Birmingham, USA).

* Author to whom correspondence should be addressed. Telephone: +86-29-84774598; fax: +86-29-83253816; e-mail: immu_jin@fmmu.edu.cn.

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All chemicals used were of analytical grade; ovalbumin (Grade VII), ovomucoid (Type III), bovine serum albumin (BSA), TMB (3,3′5,5′-tetramethylbenzidine), and ABTS (2,2′-azinobis [3-ethyl benzthizazline sulfonic acid]) were purchased from Sigma (St. Louis, Missouri). PEG 4000 (Merck, Darmstadt, Germany), nitrocellulose membrane (Amersham, Buckinghamshire, United Kingdom), chemiluminescence reagent (Roche, Indianapolis, Indiana), X-ray film (Kodak, Rochester, New York), Coomassie Brilliant Blue R-250 (Fluka, Schweiz), and Sepharose 4B (GE Healthcare, Sweden) were also used in the studies. Fetal bovine serum (FBS) was purchased from Gibco (Invitrogen Corporation, Grand Island, USA).

Apparatus. ELISA plate (Corning-Costar, New York, USA), microplate reader (Bio-Rad, Hercules, California), mini protean II apparatus (Bio-Rad, Hercules, California), and Fast Protein Liquid Chromatography (FPLC) (Amersham, Buckinghamshire, United Kingdom).

Buffers and Solutions. (1) Coating buffer: 0.05 M carbonate/biocarbonate buffer, pH 9.5; (2) washing buffer for indirect ELISA: 0.15 M phosphate buffered saline (PBS) containing 0.05% (v/v) Tween 20; (3) washing buffer for sandwich ELISA and competitive ELISA: 0.15 M PBS containing 0.1% (v/v) Tween 20; (4) dilution buffer for indirect ELISA: 0.15 M PBS containing 0.1% BSA and 0.05% (v/v) Tween 20; (5) dilution buffer for sandwich ELISA and competitive ELISA: 0.15 M PBS containing 10% FBS and 0.05% Tween 20; (6) substrate buffer: 0.1 M citrate−phosphate buffer, pH 5.0; (7) ABTS solution: 5 mg of ABTS and 20 μL of 3% H_2O_2 were dissolved in 10 mL of substrate buffer; (8) TMB solution: 500 μL of 5 mg/mL TMB and 10 μL of 3% H_2O_2 were dissolved in 10 mL of substrate buffer; (9) stop solution: 2 M sulfuric acid; (10) coupling buffer: 0.1 M $NaHCO_3/Na_2CO_3$ containing 0.5 M NaCl, pH 8.3; (11) blocking buffer for affinity chromatography column: 0.75 g glycine dissolved in 20 mL of coupling buffer; (12) elution buffer: 0.75 g of glycine dissolved in 100 mL of distilled water, HCl was added until pH reached 2.4.

Generation of Monoclonal Antibodies. Hybridoma cell lines secreting mAbs to ovalbumin were raised by conventional protocol in our laboratory (*13*). Briefly, four female BALB/c mice (8 weeks old) were selected, and each one was immunized with 20 μg of ovalbumin for three times on days 1, 30, and 40. Ten days after the third immunization, serum titers were determined by indirect ELISA. The mouse with highest serum titers was boosted with 20 μg of antigen, and three days later, splenocytes were isolated from the boosted mice and were fused with Sp2/0 murine myeloma cells in the presence of PEG 4000. The positive clones were screened by indirect ELISA with commercial ovalbumin as coating antigen and then subcloned four times by limiting dilution. MAbs were produced either from supernatants of the hybridoma cultures or from ascites of mice injected with hybridomas intraperitoneally, purified by ion-exchange chromatography column connected to a FPLC system. The immunoglobulin class and subclass of each mAb was determined using the SBA Clonotyping System following the manufacturer's recommendations.

Indirect ELISA. The wells of the ELISA plate were coated with 2 μg/mL of commercially purified ovalbumin or ovomucoid in coating buffer and incubated overnight at 4 °C. After washing three times with washing buffer, mAbs we established were serially diluted from 1:500 to 1:500 000 with dilution buffer, added to the wells (100 μL/well), and incubated for 1 h at 37 °C. After extensive washing, horseradish peroxidase (HRP) conjugated goat antimouse IgG was diluted 1:2500, and 100 μL/well was added and incubated for 45 min. After a washing step again, color development was performed by adding 100 μL of ABTS solution. The plates were incubated for 15 min at 37 °C, and the absorbance at 405 nm was detected with a microplate reader.

Preparation of Standards and Samples. Commercially purified ovomucoid was repurified by affinity chromatography and used as standard. The affinity chromatography column was prepared following the manufacturer's recommendations. Briefly, 0.5 g of CNBr-activated Sepharose 4B powder was suspended in 1 mM HCl, pH 2.4; the powder swelled immediately and was washed for 15 min with 100 mL of 1 mM HCl on a sintered glass filter. Twelve milligrams of mAb No.7 was dialysed against coupling buffer overnight, then mixed with the washed CNBr-activated Sepharose 4B and rotated end-over-end for 2 h at room temperature. Excess ligand was washed away with at least 5 gel volumes of coupling buffer, and any remaining active groups were blocked with blocking buffer for 3 h at room temperature. The gel was washed with three cycles of alternating pH, each cycle consist of a wash with 0.1 M acetate buffer, pH 4.0 containing 0.5 M NaCl, followed by a wash with 0.1 M Tris−HCl, pH 8.0 containing 0.5 M NaCl. Finally, Sepharose 4B was packed in the column. Commercially purified ovomucoid was loaded and washed with PBS as binding buffer. Repurified ovomucoid was then eluted by elution buffer and immediately dialyzed against PBS. Fresh hen's eggs were purchased from a local supermarket, and freeze-dried egg white was prepared as previously described (*14*); briefly, the egg white was separated from the egg yolk and dialyzed against distilled water overnight at 4 °C. The sugar-free egg white was freeze-dried and stored at −20 °C until use. Heat denatured ovomucoid was prepared by heating repurified native ovomucoid for 30 min at 100 °C as previously described (*8*). The concentrations of total protein in commercially purified ovalbumin, ovomucoid, repurified ovomucoid, and freeze-dried egg white were determined by BCA protein assay kit following the manufacture's instruction. Samples of commercially purified ovalbumin were diluted with dilution buffer at three concentrations (1:40 000, 1:80 000, and 1:160 000). Influenza vaccine samples produced for the 2007–2008 season and rabbit polyclonal antibody-based sandwich ELISA kit for ovalbumin were kindly provided by Sinovac Biotech Co., Ltd. (Bejing, China). The ELISA kit was constructed with polyclonal rabbit antiovalbumin antibody and its HRP-conjugated form; the procedure was the same with sandwich ELISA of ovomucoid. Influenza vaccine samples were measured after 1:5 and 1:10 dilution at the same time. Finally, mean value was calculated.

Sandwich ELISA and Method Validation. A 100 μL portion of antiovomucoid mAb No. 7 (10 μg/mL in coating buffer), as a capture antibody, was added to each well of an ELISA plate, and the plate was incubated overnight at 4 °C. The plate was washed three times with washing buffer and then blocked (200 μL/well) by adding dilution buffer. After incubation for 30 min at 37 °C, the blocking reagent was discarded, and the plate was tapped over adsorbent paper. For the standard curve, repurified ovomucoid was serially diluted with dilution buffer; then, 100 μL/well samples and standards were added to the wells, respectively. After incubation for 1 h at 37 °C and washing five times, the wells were reacted with a detecting antibody, antiovomucoid mAb No. 9 (2.8 μg/mL) that was conjugated with HRP and diluted 1:500 in dilution buffer. The plate was incubated for 1 h at 37 °C and washed six times; 100 μL/well of substrate solution containing TMB was added, and the plate was incubated for 25 min at room temperature in a dark chamber. The reaction was subsequently quenched with 2 M sulfuric acid (50 μL/well), and the absorbance at 450 nm was measured with a microplate reader.

Intra-assay variation was determined with repurified ovomucoid at three concentrations using seven replicates of each concentration on the same ELISA plate, the assay was repeated three times. Interassay variation was estimated on 10 different assays in triplicate, performed on different days by the same operator. Diluted egg white and influenza vaccine samples were spiked with different amounts of ovomucoid (0.1–3.12 ng/mL) for recovery studies. Recoveries were calculated according to the following eq,

$$R(\%) = \frac{[OM_t - (OM_{EW} \text{ or } OM_{IV})]}{OM_a \times 100} \quad (1)$$

where OM_{EW} or OM_{IV} is the ovomucoid concentration measured in diluted egg white or influenza vaccine, respectively, OM_a is the concentration of ovomucoid added in egg white or influenza vaccine, and OM_t is the total concentration of ovomucoid measured in spiked diluted egg white or influenza vaccine. Cross-reactivity was further investigated by testing bovine serum, horse serum, goat serum, human serum, and chicken serum with no dilution and duck, goose, quail, and pigeon egg white diluted 1:100.

Competitive ELISA. A 100 μL/well portion of 0.02 μg/mL repurified native ovomucoid was coated to an ELISA plate overnight

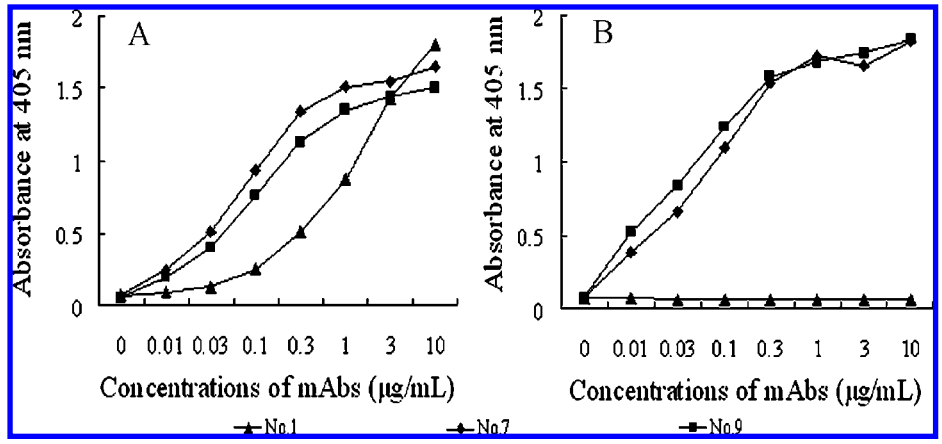

Figure 1. Reactivity of mAbs against commercially purified ovalbumin (**A**) and ovomucoid (**B**) identified by indirect ELISA. The same results were obtained as mAb No.1 when mAbs No. 2–6 and No. 8 were applied in this indirect ELISA.

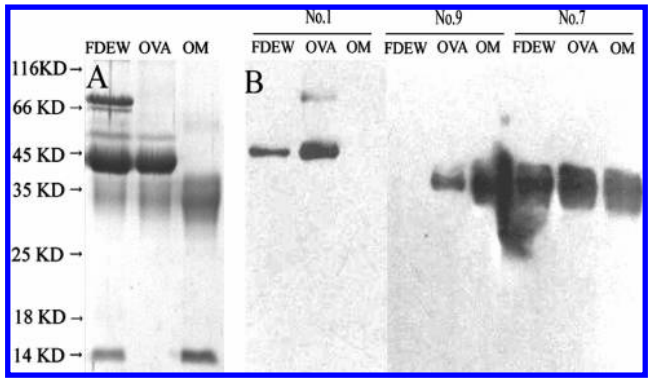

Figure 2. Specificity of antiovalbumin or ovomucoid mAbs determined by SDS-PAGE and Western blot. (**A**) SDS-PAGE analysis of freeze-dried egg white (FDEW), commercially purified ovalbumin (OVA) and ovomucoid (OM). Ten micrograms of each protein were applied to the gel and directly stained with Coomassie Brilliant Blue. (**B**) Specificity of mAbs determined by Western blot. One microgram of ovalbumin, ovomucoid, or FEDW were loaded to the corresponding lane on the gels (4 μg of ovalbumin and ovomucoid for mAb No. 9). The same results were obtained as mAb No. 1 when mAbs No. 2–6 and No. 8 were applied in Western blot.

at 4 °C. The plate was washed three times with washing buffer. Afterward, free binding sites of the wells were blocked with 200 μL/well of dilution buffer for 1 h at room temperature to prevent unspecific bindings of the antibody. The plate was washed three times with washing buffer; 75 μL/well of native or heat denatured ovomucoid diluted serially and 50 μL/well of HRP-conjugated mAb No. 7 or No. 9 (diluted 1:4000) were added to the coated wells in succession. After 1 h of incubation at 37 °C, the plate was washed as before, 100 μL/well of substrate solution containing TMB was added, and the plate was incubated for 15 min at room temperature in a dark chamber. The reaction was quenched with 2 M sulfuric acid (50 μL/well), and absorbance at 450 nm was measured with a microplate reader. Calibration curves were constructed in the form of $B/B_0 \times 100\%$ vs log C, where B and B_0 was the absorbance of the analyte at added and zero concentrations, respectively.

SDS-PAGE and Western Blot. Freeze-dried egg white, ovalbumin, and ovomucoid were analyzed on 4–12% SDS acrylamide gels with mini protean II apparatus. Separation was performed under reducing conditions at constant voltage 180 V for 40 min. The proteins were either stained with Coomassie Brilliant Blue R-250 or electrophoretically transferred from the gel onto a nitrocellulose membrane for Western blot. The membrane was blocked in 5% nonfat milk and incubated with culture supernatant of antiovomucoid or ovalbumin clones separately at 4 °C overnight. After a 30 min rinse, the membrane was reacted with HRP-labeled goat antimouse IgG polyclonal antibody for 1 h at room temperature. Following another 30 min rinse, enhanced chemiluminescence reagent was applied to the membrane, which was then exposed to X-ray film.

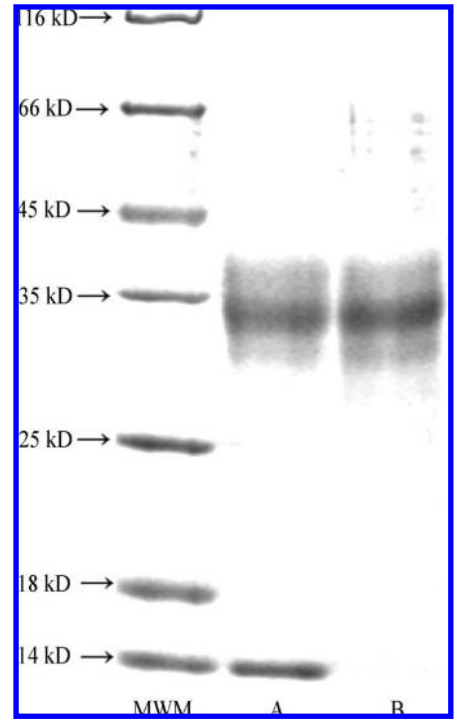

Figure 3. SDS-PAGE analysis of standard purity. Lane A, 10 μg of commercially purified ovomucoid; lane B, 10 μg of affinity chromatography repurified ovomucoid.

RESULTS

Identification of mAbs to Ovomucoid. Nine positive hybridoma clones secreting mAbs to commercially purified ovalbumin were obtained and designated No. 1–9. Interestingly, two clones, No. 7 and No. 9, reacted to both commercially purified ovalbumin and ovomucoid, whereas others failed to recognize ovomucoid (**Figure 1**). This indicated that commercially purified ovalbumin was contaminated with ovomucoid. SDS-PAGE analysis supported this presumption (**Figure 2A**); the image of SDS-PAGE showed that the main contaminant in commercially available ovalbumin was ovomucoid (Apparent MW 30–43 kDa) (*3, 10, 15*). Subsequently, Western blot further confirmed that both No. 7 and No. 9 mAbs recognized ovomucoid in commercial ovalbumin, ovomucoid, and freeze-

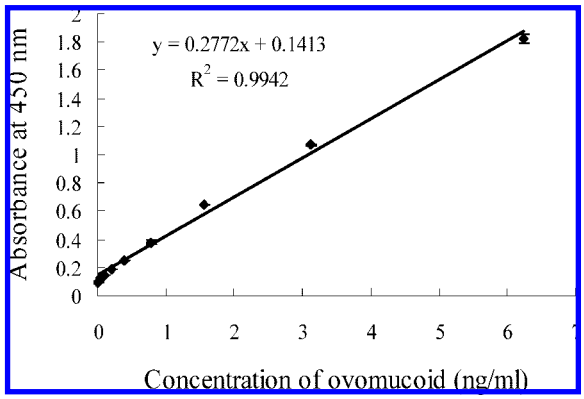

Figure 4. Standard curve for ovomucoid. Affinity chromatography repurified ovomucoid was used as standard. Points represent the mean absorbance values of duplicate wells. Where not visible, the error bars are within the symbol.

Table 1. Determination of Ovomucoid in Influenza Vaccine Samples by Quantititive Sandwich ELISA

influenza vaccine	ovomucoid (ng/dose[a])	ovalbumin (ng/dose)
sample 1	7.61	49.85[b]
sample 2	5.20	28.89[b]
sample 3	3.67	23.72[b]
sample 4	9.98	43.53[b]

[a] The volume of one dose is 0.5 mL. [b] Detected by polyclonal antibody-based ELISA specific for ovalbumin.

dried egg white, while No. 1 mAb reacted to ovalbumin (**Figure 2B**). The apparent molecular weight of ovomucoid appeared larger by SDS-PAGE than that caculated from its molecular constituents, and the band shape of ovomucoid was broad; this may be due to the high content and spatial distribution of carbohydrate along the polypeptide chain of ovomucoid (*3, 5, 10*).

Establishment of Sandwich ELISA Specific for Ovomucoid. Using mAb No. 7 as capture antibody and HRP-conjugated mAb No. 9 as detection antibody, we established a sandwich ELISA kit specific for ovomucoid. Repurified ovomucoid was used as standard, the purity was at least 97% analyzed by the SDS-PAGE image using software (BandScan, version 5.0) (**Figure 3**). The low detection limit reached 0.041 ng/mL, which was defined as the lowest ovomucoid concentration outside the range of three standard deviations over negative control (n = 21). The linear dynamic range was between 0.1 and 6.25 ng/mL (**Figure 4**). Precision was determined by inter- and intra-assay coefficients of variation (CVs); interassay CVs were 2.35% at 5.0 ng/mL, 3.12% at 2.5 ng/mL, and 4.23% at 0.5 ng/mL, and intra-assay CVs were 1.80% at 5.0 ng/mL, 2.42% at 2.5 ng/mL, and 3.65% at 0.5 ng/mL, respectively. Recovery studies showed that when egg white was diluted 1: 3 200 000 to 1:50 000 000 and the ratio of OM_a/(OM_{EW} or OM_{IV}) was larger than one-third (concentrations of OM_a ranged from 0.2 to 3.12 ng/mL), then recoveries ranged from 91.7 to 104.3%; low recovery of added ovomucoid was obtained (≤81%) when the concentrations was lower or equal to 0.1 ng/mL. When influenza vaccine sample was diluted 1:5 (OM_{IV} was measured at 1.6 ng/ml) and spiked with 1 ng/mL of ovomucoid, the recovery was 98.7%. The ELISA kit has no cross-reactivity against bovine serum, horse serum, goat serum, and human serum, but had a low level of reactivity to chicken serum (data not shown). Importantly, only ovomucoid from hen's egg white could be detected but not duck, goose, quail, or pigeon egg white. Interestingly, mAb No. 7 reacted with duck and goose egg white equally to hen's egg white, but could not react with quail and pigeon egg white, while mAb No. 9 could only recognize ovomucoid from hen's egg white (data not shown). These characteristics of mAbs No. 7 and No. 9 indicate that ovomucoid from hen's egg white not only contains unique epitopes of its own but also shares a common epitope(s) with other bird eggs.

Detection of Ovomucoid in Commercially Purified Ovalbumin. First, we determined the percentage of ovomucoid in freeze-dried egg white by the sandwich ELISA kit we established; the result (9.6%) was consistent with the data previously reported (*8, 15, 16*). Then, the contamination degree of ovomucoid in commercially purified ovalbumin samples were quantitatively measured. Sample values (Grade VII) of two different lots were similarly calculated from the linear regression equation, and the mean value was 11% of total proteins. We got similar result from the image of SDS-PAGE (**Figure 2A**), in which the amount of samples to each lane were all 10 μg, and the bands of ovomucoid in ovalbumin and freeze-dried egg white protein were comparable.

Detection of Ovomucoid in Influenza Vaccine Samples. Influenza vaccines contained varying amount of residual egg proteins because it was produced in embryonated hen's eggs (*17*). Although ovomucoid was thought to be the major allergen in egg white, it had not been evaluated in influenza vaccines

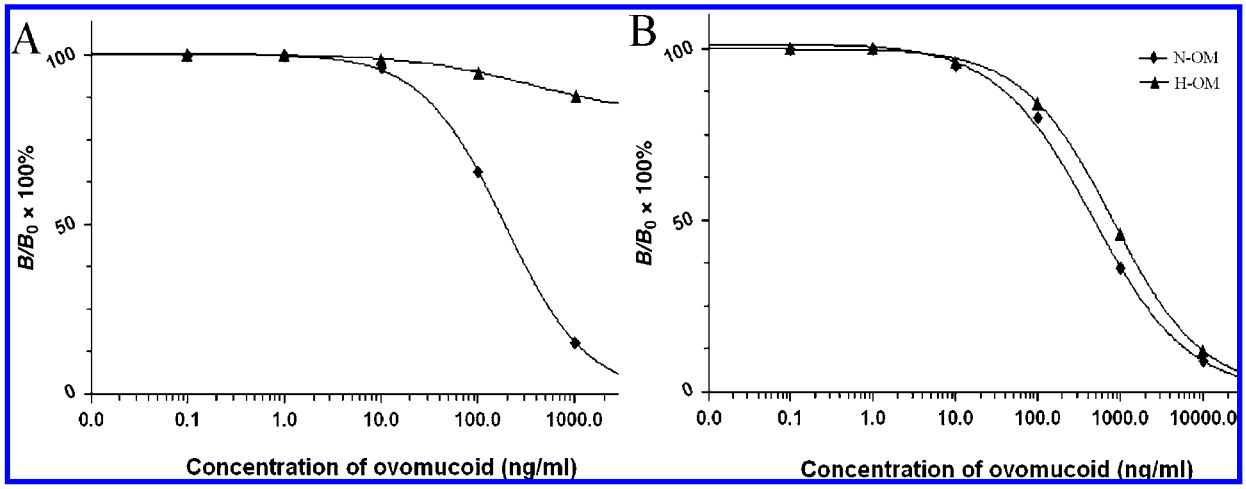

Figure 5. Recognition of mAbs No. 9 (**A**) and No. 7 (**B**) to native (N-OM, ♦) or heat denatured (H-OM, ▲) ovomucoid determined by competitive ELISA. B and B_0 were the absorbance of the analyte at added and zero concentrations, respectively.

because of the limited sensitivity and specificity of previous methods. Here, we measured the amount of ovomucoid in four influenza vaccine samples and compared them with the amount of ovalbumin detected by polyclonal antibody-based sandwich ELISA (**Table 1**). The results showed that there were a certain amount of ovomucoid held in the influenza vaccine samples, and interestingly, the ratio of ovomucoid to ovalbumin was about 1/5, similar to hen's egg white.

Application to Food Products. It was previously reported that there were two forms of ovomucoid: native form (N-OM) and heat denatured form (H-OM) (*8*). Different forms of ovomucoid could be recognized by different mAbs. Therefore, we analyzed the reactivity of mAb No. 7 and No. 9 against the heat denatured form of ovomucoid by competitive ELISA. The binding of mAb No. 9 to the fixed N-OM could hardly be inhibited by free H-OM as compared with N-OM (**Figure 5A**); however, the inhibition effects induced by H- and N-OM to the binding of mAb No. 7 were comparable (**Figure 5B**). Lower than 10 ng/mL ovomucoid could be detected. These results indicate that the sandwich ELISA could only be used for unprocessed food products, whereas mAb No. 7-based competitive ELISA could be a potent method for the detection of egg white allergen in processed food products.

DISCUSSION

Commercially available ovalbumin was widely used in basic and clinical research as a model antigen (*10*). However, the contamination of ovomucoid has led to an overestimation of the dominance of ovalbumin as the major egg allergen (*3, 5, 10*). Therefore, it is crucial for researchers to quantitatively identify the degree of ovomucoid contamination in ovalbumin. In the previous studies, SDS-PAGE followed by silver staining and Western blot have been applied to reveal the degree of contamination, respectively (*3, 10*); the mAbs-based ELISA kit has been established for the detection of food samples (*8*). Wang et al. determined the purities of two ovalbumin samples, grade V and II, using SDS-PAGE on the basis of gel image analysis; the values were 76.0 and 38.0%, respectively (*11*). Mine et al. also reported that there was approximately 6.7% impurity in the commercially available ovalbumin as calculated from the HPLC profile, and the impurity was identified as ovomucoid by analyzing with SDS-PAGE and amino acid sequencing (*12*). In the present work, using mAbs generated by commercially available ovalbumin, we have established an ELISA kit specific for ovomucoid from hen's egg white. As compared with the methods reported previously, it was more sensitive and simple. The use of mAbs ensured its specificity. These characteristics make it possible for the detection of ovomucoid precisely in basic and clinical research.

There were contradictory results previously reported in the studies on the relative allergenicity and antigenicity of egg white protein, one explanation for these discrepancies was the lack of purity of the egg white protein used in various studies (*7*). With the use of highly purified egg white protein, ovomucoid was gradually proved to be the major egg white allergen (*5, 7*). However, we found that in many studies the contamination degree of ovomucoid in commercially purified ovalbumin had not been precisely measured when they were used as comparison with the highly purified ovalbumin. For example, Bernhisel-Broadbent et al. originally evaluated the allergenicity and antigenicity dominance of hen's egg ovomucoid. In their studies, the impurity of commercially purified ovalbumin was considered to be less than 1% without further identification (*5*). Our data showed that there was 11% of ovomucoid contaminant in commercially purified ovalbumin samples; therefore, it was difficult to estimate the extent of immunodominance induced by ovomucoid depending on the data in literatures. We believe that the immunodominant status of ovomucoid needs to be further described.

The reaction pattern of mAbs No. 7 and No. 9 to bird eggs may be served as one of the reasons for clinically identified allergy case reacting to different bird eggs (*18, 19*). We evaluated ovomucoid levels in influenza vaccine for the first time, and our data showed that the amount of ovomucoid was about 1/5 of the ovalbumin in influenza vaccine samples. Ovomucoid could be an effective marker for the detection of egg residues in vaccines derived from embryonated hen's eggs, because the detection limit was lower than ovalbumin and because ovomucoid was one of the major allergens in egg white. We also discussed the application of the ELISA methods for detecting food products. Only unprocessed food could be detected by sandwich ELISA because of the reactivity characteristic of mAb No. 9. Another mAb, No. 7, could recognize ovomucoid from hen's egg white, duck egg white, and goose egg white, but it had no reactions with quail egg white and pigeon egg white. Importantly, mAb No. 7 reacted with native and heat denatured ovomucoid almost equally, so the mAb No. 7-based competitive ELISA could be further developed into indirect competitive ELISA and amplified for the detection of processed foods.

In conclusion, an ELISA kit with high sensitivity and specificity was established and can be used as an effective method for the quantification of ovomucoid from hen's egg white.

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