Nutraceuticals & Food
Vol. 7, p.373~377 (2002)

Nutraceuticals and Food

Determination of Ovalbumin in Processed Foods by Immunological Methods

Ji-Hyun Seo, Ju-Woon Lee, Sin-Bok Kang*, Ha-Baik Lee**, Hong-Sun Yook***, Mee Ree Kim***, Kee-Hyuk Kim**** and Myung-Woo Byun†

The Team for Radiation Food Science and Biotechnology, Korea Atomic Energy Research Institute, Daejeon 305-600, Korea
**Department of Communication and Information, Korea Atomic Energy Research Institute, Daejeon 305-600, Korea*
***Department of Pediatrics, School of Medicine, Hanyang University, Seoul 133-792, Korea*
****Department of Food and Nutrition, Chungnam National University, Daejeon 305-764, Korea*
*****School of Food Science and Biotechnology, Woosong University, Daejeon 300-718, Korea*

Abstract

Allergens in processed foods may place persons with food allergies at significant risk when the labels do not provide sufficient warnings or identification of high-risk ingredients. Because egg proteins are common food allergens, this study was carried out to identify hen's egg albumin (ovalbumin, OVA) in five commercially processed foods containing egg (custard, cookie and pasta), and chicken meat (sausage and meatball) by immunological methods using commercially produced murine monoclonal immunoglobulin G (M-IgG), immunoblotting and enzyme linked immunosorbent assay (ELISA). Sample buffer with chelating and reducing agents was prepared and used for the preparation of the protein fractions from the foods. Most bands in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) profile (5~15% gradient gel) presented at 75 kDa below. OVA (43 kDa) in the sample lanes could not be visually observed on the gel. However, OVA in solutions prepared from custard and cookie could be detected by M-IgG, but were not detected in sausage and pasta. OVA in all samples could be quantitatively determined by the equation obtained from the standard curve by ELISA. Cookie and custard containing egg white and egg, respectively, contained very high concentrations of OVA. OVA in the other products were present in relatively low concentrations, but sufficiently high to pose possible risk of allergy. ELISA is a very sensitive and precise method for the identification and quantification of allergens in food products including allergy-inducible materials.

Key words: ovalbumin, allergen, immunoassay

INTRODUCTION

Egg is widely used as an ingredient in the production of bread, cookie and cake, noodle and pasta, meat products, etc, for specific applications such as flavor enhancement, gelation, foaming action, thickening, and emulsification (1). However, egg white is one of the most prevalent allergens responsible for food hypersensitivity reactions, particularly in atopic children (2). Egg-white constituents appear to be excellent indicators for the assessment of masked allergens in processed foods (3-5). Egg white contains about 40 different proteins, some of which have been shown to be major allergens, including: ovalbumin (OVA), ovotransferrin, ovomucoid and lysozyme (6). OVA (54% of whole egg white) is the most extensively studied allergen having been subjected to considerable basic research as a model allergen (7,8). Hidden allergens can be present in processed food when an allergenic ingredient is added, but not disclosed on the label, thereby presenting a potential risk for allergic consumers (9,10). Detection of the individual constituents of modern food products can be difficult due to low concentrations of allergens, or treatments involved in their production which can modify allergen potency. It is important to assess the allergenicity and prevalence of masked allergens for the protection of food-allergic consumers (5).

Chicken meat is sometimes added to processed meat products to reduce production cost while maintaining high quality protein values in the final product (11). Because chicken albumin is derived from egg albumin, patients with either chicken or egg allergy may have cross-reactions with the other (12); safety against egg allergy should also be ensured in the products containing chicken meat.

Although many processed foods containing egg are domestically produced, many products contain no notice phrases such as "May be allergy-induced by egg or/and chicken meat" on the label with the other product information such as nutrition values. Unfortunately, egg allergy reac-

†Corresponding author. E-mail: mwbyun@kaeri.re.kr
Phone: +82-42-868-8065, Fax: +82-42-868-8043

tions caused by the intake of processed foods, without warnings, are frequently reported by medical associations (13), therefore cautionary label warnings are needed to prevent allergic reactions from the ingestion of these products by allergic individuals.

Several studies have been conducted to determine egg or egg white content of food products by the application of electrophoresis, chromatography and immunochemical methods (14,15). Immunochemical approaches can be precisely and rapidly used to identify egg proteins added to processed food (16,17).

In this study, immunoblot and competitive indirect enzyme linked immunosorbent assay (Ci-ELISA) methods, using a commercially produced murine monoclonal antibody (Ab) against OVA as a model antigen (Ag), were evaluated for use as preliminary assays for rapid and precise determination of egg allergens in domestically sold foods that have no warning notification of egg allergen ingredients.

MATERIALS AND METHODS

Protein and antibodies

Isolated hen's egg albumin (OVA) was purchased from the Sigma Chemical Co. (St Louis, MO, USA) to use as a standard Ag of egg white protein. OVA was dissolved in 0.01 M sodium phosphate buffered saline (PBS) with 0.15 M NaCl, pH 7.4 and adjusted to a final concentration of 2.0 mg/mL. Murine monoclonal immunoglobulin G (M-IgG) against OVA was also purchased from the Sigma Chemical Co. Horseradish peroxidase (HRP) conjugated to rabbit anti-mouse IgG (Sigma Chemical Co.) was used as secondary Ab with a chromogenic enzyme (tracer).

Sample preparation

Custard, pasta, cookies, comminuted sausage, and meatballs were purchased from a domestic market and used in the subsequent experiments. The approximate compositions of the products are shown in Table 1.

Two-gram sample was homogenized in extraction buffer (0.1 M PBS, pH 7.0 containing 6 M urea, 0.01 M ethylenediaminetetra acetic acid disodium salt and 0.01 M dithiothreitol) as the previous research (16) for 2 min.

The homogenate was stirred at 4°C for 4 h and centrifuged at 9,000×*g* for 30 min. The supernatant was filtered through a 0.45 μm filtration kit (MILLEX^{R}-HV, Millipore, Molsheim, France) and dialyzed in a cellulose tube (pore size <5,000, Spectrum^{R} Medical Industries, Inc., Houston, TX, USA) in 0.01 M PBS. After dialysis, the protein concentration of the sample solutions were determined using a bicicinchonic acid protein assay kit (Sigma Chemical Co.) with bovine serum albumin (BSA) solution as a standard and a spectrophotometer (UV-1600PC, Shimadzu Corp., Kyoto, Japan) set at 562 nm, as the previously described method (18).

SDS-PAGE and immunoblot

SDS-PAGE (5~15% gradient gel) for sample solutions (2.0 mg/mL) was performed by Laemmli's method (19) using a Hoefer vertical electrophoresis apparatus SE-600 (Pharmacia Biotech., Uppsala, Sweden), and a staining and destaining method on a gel as the previously described method (20). A prestained molecular weight marker was purchased from Bio-Rad Laboratories (Hercules, CA, USA) to determine the molecular weights. The marker consisted of myosin (208 kDa), β-galactosidase (115 kDa), BSA (79.5 kDa), OVA (49.5 kDa), carbonic anhydrase (34.8 kDa), soybean trypsin inhibitor (28.3 kDa), lysozyme (20.4 kDa) and aprotinin (7.2 kDa).

Proteins on another gel electrophoretically separated under the same conditions were transferred onto a nitrocellulose paper (pore size <0.45 μm, Sigma Chemical Co.) by the previously described method (21), and the immunoblotting with M-IgG was performed in a rotary incubator (Techne Ltd., Duxford, Cambridge, UK) by the induction procedure for immune reaction between M-IgG and OVA using the same ELISA conditions, except that the dilution rates were 1:1,000 for M-IgG and 1:5,000 for the tracer, and 4-chloro 1-naphtol (Sigma Chemical Co.) was used as the chromogen.

Ci-ELISA

Ci-ELISA was formatted with M-IgG by the method reported previously (22). Briefly, polystyrene flat-bottom microtiter plates (Maxisorp, Nunc, Kamstrup, Denmark) were coated with 100 μL of OVA solution (1.0 μg/mL) in a 0.2 M bicarbonate buffer, pH 9.6, overnight at 4°C. All subsequent steps were performed at 37°C. Plates were washed

Table 1. The approximate compositions of the processed foods used in this experiment

Products	Raw materials (content)	Remark
Custard	Flour, shortening, white sugar, egg, isomaltose oligomer	No indication of the content of materials
Cookie	Flour, shortening, white sugar, egg white, powdered skim milk	
Pasta	Durum wheat semolina	
Sausage	Chicken meat (45.04%), pork (27.44%), starch, frozen gluten, isolated soy protein, calcium sorbate, sodium nitrite, sodium erithrobate, coloring agent	Partial indication of the content of materials
Meatball	Pork (46.37%), chicken (17.53%), beef (9.26%)	

three times with 0.01 M PBS containing 0.05% (v/v) Tween 20 (PBST). To reduce non-specific binding, the plates were blocked by incubation for 2 h with 120 μL of PBS containing 1% (w/v) BSA. After washing, 50 μL of either standard or sample solutions were added to coated and blocked wells, and then 50 μL of Ab solutions were added. The plates were incubated for 2 h, and then washed three times with PBST. After the addition of 100 μL of tracer solution to the wells, the plates were incubated for 2 h. The plates were then washed, and 100 μL of 0.04% *o*-phenylenediamine (Sigma Chemical Co.) in 0.1 M phosphate-citrate buffer, pH 5.0 with 0.04% hydrogen peroxide (v/v, 35% H_2O_2) was added to produce the color reaction for 20 min before stopping the reaction with 2.0 M H_2SO_4 (50 μL/well). The absorbance was measured at 492 nm using an ELISA reader (CERES UV-900C, BIO-TEK Instruments Inc., New York, NY, USA).

For standards, OVA solution was serially prepared in concentrations from 3.9 μg/mL to 4,000 μg/mL. Sample solutions were also serially diluted to 32 times in PBS. M-IgG and tracer were diluted 10,000 times and 20,000 times, respectively in PBS.

The concentration of OVA in diluted sample solution was determined using a standard curve derived from the reaction of M-IgG to OVA in Ci-ELISA.

Statistical analysis

All samples were done in triplicate and the experiments were repeated 5 times. The means and standard deviations were used to evaluate the detected concentration of OVA calculated from the equation of M-IgG. The data were analyzed by general linear procedures and least square means of SAS® (SAS Institute, Cary, NC, USA) (23).

RESULTS AND DISCUSSION

SDS-PAGE profile and immunoblot

SDS-PAGE profiles (5~15% gradient gel) of proteins in sample solutions are shown in Fig. 1. Most bands on the gel represent proteins at about 75 kDa below. OVA (43 kDa) in the lanes of samples could not be seen on the gel. However, OVA in the molecular weight marker lane and in solutions prepared from custard and cookie could be detected by immunoblotting with M-IgG, even though the results were unable to be seen on a photo due to weak binding reaction (Fig. 2). OVA were not detected in samples of sausage and pasta. A sample solution prepared from meatballs was loaded onto a gel and electrophoretically separated, but yielded poor quality results that could not be interpreted (data not shown), therefore immunoblot to the gel was not performed.

In general, SDS-PAGE has been widely used for the determination of protein profiles in protein samples (19).

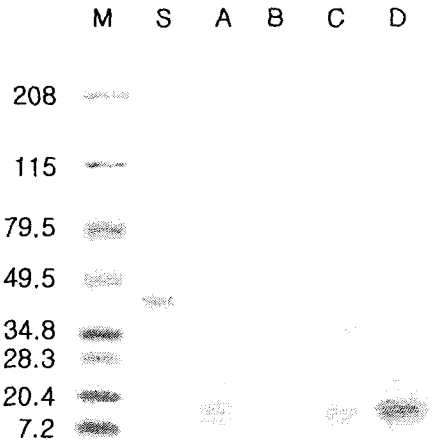

Fig. 1. SDS-PAGE profile of proteins in sample solutions. M, Prestained molecular weight standard marker; S, standard ovalbumin; A, Custard; B, Cookie; C, Sausage; D, Pasta. Numbers on the left side indicate the molecular weights (kDa) of the standard marker.

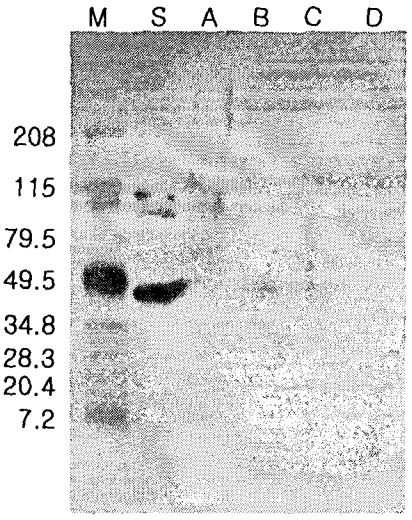

Fig. 2. Immunoblot of ovalbumin in sample solutions by murine monoclonal IgG. M, Prestained molecular weight standard marker; S, standard ovalbumin; A, Custard; B, Cookie; C, Sausage; D, Pasta. Numbers on the left side indicate the molecular weights (kDa) of the standard marker.

However, it is not sensitive enough to detect very small amounts (< 1.0 μg/mL) of target proteins, even if the silver-staining method is used (6). Furthermore, some proteins cannot be positively identified because of the existence of proteins with the same molecular weight. This problem can be solved by using blotting techniques employing specific Ab. Ab can recognize a specific site on an Ag, and this recognition appears the chromogenic result by the reaction with the Ag on a paper transferred from a gel (21).

Quantification of OVA in sample solutions by Ci-ELISA

A standard curve was obtained for quantifying the OVA

in a sample solution (Fig. 3). The curve, which appeared as a sigmoid line, could be generally obtained from a semi logarithmical function as previously reported (24,25). OVA could be quantitatively determined in the range of 7.8 to 500.0 μg/mL and the concentration could be calculated by the equation ($X = e^{((3.1636-Y)/0.4331)}$) derived from the curve: where X is the concentration of OVA in sample solutions and Y is the average OD value of the microwells of a sample solution. The ELISA formatted in this study could be applied to determine small amounts (1 μg/g sample) of OVA in samples.

Table 2 shows the concentrations of OVA in sample solutions prepared from processed foods including egg or chicken meat by Ci-ELISA. Cookie and custard, made with egg white and whole egg, respectively, had a very high OVA content. All other products had relatively low concentrations of OVA.

Because a very small amount of allergen, less than 10 ~20 ng/g, can induce egg hypersensitivity reactions (2,4), and all of the products we tested had that much or more, it is important that labels clearly indicate all ingredients and provide warnings of possible allergic reactions. However, patients with egg allergies are frequently unable to obtain the necessary ingredient information (Table 1) and are exposed to the risk of serious, possibly life-threatening, allergic reactions (13).

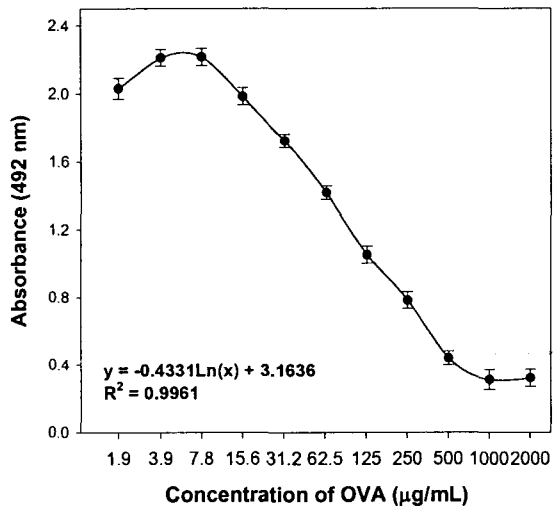

Fig. 3. Standard curve for quantifying ovalbumin (OVA). The curve was made by Ci-ELISA formatted with murine monoclonal IgG (M-IgG). Standard solution was prepared by the dilution of ovalbumin solution (4.0 mg/mL in PBS buffer, pH 7.4). The equation in figure was used to determine the amount of OVA in sample solution recognized by M-IgG. X and Y indicate the concentration of OVA and OD value obtained from immune reaction of M-IgG and OVA coated onto microwell, respectively.

We searched domestic food product information data for consumer warnings or declarations indicating ingredients that might cause food allergy or food hypersensitivity reactions on food labels, on- and off-line databases provided by manufacturers, library sources, and associations and related organizations over several years. However, none of the sources we searched provided consumer warnings or educational material related to food allergy.

Pasta used in this study, in which OVA was detected, only listed the major ingredient, durum wheat semolina. It is especially important for imported food items, such as pasta for which the domestic population may be unfamiliar with traditional ingredients, to be carefully regulated for product ingredient labeling. Labeling laws requiring allergy warnings for foods containing high-risk ingredients should be enacted as a means of protecting public health.

Analytical methods using immunological approaches are increasing used, due to the rapid and precise results for identification and quantification of allergens in food products, including allergy-inducible materials (2,6,15). Immunological assays can be used more effectively by utilizing serial procedures for targeting Ag with epitopes durable to processing, and employing various species of monoclonal or polyclonal Ab produced against target Ag, and using them for designing novel analytical methods. More correct results can be obtained by the use of solid phase techniques, such as ELISA used in this study, radioimmunoassay, chemiluminescence immunoassay or fluoroscence immunoassay than by the use of immunoblot or of liquid phase techniques such as immunodiffusion and immunorocket methods that were developed initially (3,7). Furthermore, the use of monoclonal rather than polyclonal Ab is recommended due to the greater precision in identifying certain Ag/allergens in foods (25). However, when monoclonal Ab are used, the property of Ag should be considered and evaluated. For example, ovomucoid has been described as noncoagulable by heat and is not denatured by 8 M urea, whereas OVA is thermolabile (26,27). In contrast, the heat stability of the other allergens has not been studied in detail. Because most processed foods are manufactured by processing treatments that include changes in pH and/or ionic strength, freezing and heating, etc, that can denature Ag, the choice of the epitope (Ag-determinant

Table 2. Detected concentrations of ovalbumin in sample solutions prepared from commercially processed foods by Ci-ELISA formatted with mouse murine monoclonal IgG unit (μg/mL)

	Custard	Cookie	Pasta	Sausage	Meatball
OVA content	43,184.1±2,182.7[1)]	68,219.2±3,697.6	554.0±114.5	422.6±83.4	284.6±113.8

[1)]Mean±SD (n=5).

ite) is very important in the production of monoclonal Ab 2).

In conclusion, the immunological approach can be effectively used as a preliminary analysis for the detection of egg allergens in processed foods.

ACKNOWLEDGEMENTS

This project has been carried out under the Nuclear Research & Development Program by the Korean Ministry of Science and Technology.

REFERENCES

1. Mine Y. 1995. Recent advances in the understanding of egg white protein functionality. *Trends Food Sci Technol* 6: 225-231.
2. Langeland T. 1982. A clinical and immunological study of allergy to hen's egg white. III. Allergens in hen's egg-white studied by crossed radio-immunoelectrophoresis (CRIE). *Allergy* 37: 521-530.
3. Steinman HA. 1996. "Hidden" allergens in foods. *J Allergy Clin Immunol* 98: 241-250.
4. Sampson HA, Mendelson L, Rosen JP. 1992. Fatal and near-fatal anaphylactic reactions to food in children and adolescents. *N Engl J Med* 327: 380-384.
5. Moneret-Vautrin DA. 1995. Masked food allergens. In *Intestinal immunology and food allergy*. de Weck AL, Sampson HA, eds. Nestle Nutrition Workshop Series, Nestle Ltd. Raven Press, New York. Vol 34, p 249-257.
6. Holen E, Elsayed S. 1990. Characrization of four major allergens of hen egg-white by IEF/SDS-PAGE combined electrophoretic transfer and IgE-immunoautoradiography. *Int Arch Allergy Appl Immunol* 91: 136-141.
7. Miller H, Campbell DH. 1950. Skin test reactions to various chemical fractions of egg white and their possible clinical significance. *J Allergy* 21: 522-524.
8. Anet J, Back JF, Baker RS, Barnett D, Burley RW, Howden MEH. 1985. Allergens in the white and yolk of hen's egg. *Int Arch Allergy Appl Immunol* 77: 364-371.
9. Hoffman DR. 1983. Immonochemical identification of the allergens in egg white. *J Allergy Clin Immunol* 71: 481-486.
10. Fremont S, Kanny G, Bieber S, Nicolas JP, Moneret-Vautrin DA. 1996. Identifiaction of a masked allergen, α-lactalbumin, in baby-food cereal flour guaranteed free of cow's milk protein. *Allergy* 51: 749-754.
11. Smith DM. 1995. Immunoassays in process control and speciation of meats. *Food Technol* 49: 116-119.
12. Cahen YD, Fritsch R, Wuthrich B. 1998. Food allergy with monovalent sensitivity to poultry meat. *Clin Exp Allergy* 28: 1026-1030.
13. Nam SY, Lee SI. 2000. Therapy and prevention of food allergy. *Food Sci Ind* 33: 16-21.
14. Mikes C. 1988. *High performance liquid chromatography of biopolymers and biooligomers*. Part B. Elsevier, Amsterdam. p 87.
15. Leduc V, Demeulemester C, Polack B, Guizard C, Le Guern L, Peltre G. 1999. Immunochemical detection of egg-white antigens and allergens in meat products. *Allergy* 54: 464-472.
16. Sajdok J, Rauch P, Paluska E, Kas J. 1990. Determination of egg and egg white content of food products by means of immunochemical assessment of ovalbumin. *J Sci Food Agric* 53: 253-259.
17. Enrique E, Cistero-Bahima A, Alonso R, San Miguel MM. 2000. Egg protein: a hidden allergen in candies. *Ann Allergy Asthma Immunol* 84: 636.
18. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC. 1985. Measurement of protein using bicinchoninic acid. *Anal Biochem* 150: 76-85.
19. Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680-685.
20. Claeys E, Buts UR, Demeyer D. 1995. Quantification of beef myofibrillar proteins by SDS-PAGE. *Meat Sci* 39: 177-193.
21. Towbin H, Staehelin T, Gordon J. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc Natl Acad Sci USA* 76: 4350-4354.
22. Lee JW. 1999. Application of immunoassay for monitoring conformational changes of myosin under physico-chemical treatment. *PhD dissertation*. Kon-Kuk University, Korea
23. SAS. 1989. *SAS/STAT User's Guide*. Version 6.12. SAS Institute Inc., Cary, NC.
24. Lee JW, Yook HS, Lee HJ, Kim JO, Byun MW. 2001. Immunological assay to detect irradiated beef. *J Food Sci Nutr* 6: 91-95.
25. Lee JW, YooK HS, Cho KH, Cha BS, Byun MW. 2001. Application of immunoassay for the detection of gamma-irradiated shrimp. *J Korean Soc Food Sci Nutr* 30: 600-604.
26. Deutsch HF, Morton JI. 1956. Immunochemical properties of heated ovomucoid. *Arch Biochem Biophys* 64: 19-25.
27. Mine Y, Zhang JW. 2002. Comparative studies on antigenicity and allergenicity of native and denatured egg white proteins. *J Agric Food Chem* 50: 2679-2683.

(Received September 23, 2002; Accepted October 25, 2002)