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Changes of the Binding Abilities of Immunoglobulin G and E on Gamma-Irradiated Ovalbumin by Proteolytic Enzymes

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Abstract This study evaluated the binding abilities of rabbit anti-ovalbumin (OVA) immunoglobulin G (IgG) and egg-allergic patient IgE on gamma-irradiated OVA during proteolysis using pepsin and trypsin. The concentrations of both the intact and the irradiated OVAs decreased during proteolysis when detected with IgG. However, when detected by patient IgE the concentration of the intact OVA decreased up to 30 min after the trypsin treatment and increased thereafter. Irradiated OVA detected by patient IgE showed a lower initial concentration (0.16%) than that of the intact OVA, and this reduced concentration was maintained stably. The results indicate that irradiation, rather than enzymatic treatment, could reduce the binding of the irradiated and enzyme-treated OVA. Therefore, gamma irradiation has potential as an effective method to reduce OVA-induced allergy and may enhance the safety of egg-allergic individuals.

Key words: ovalbumin, gamma irradiation, proteolysis, IgE-binding ability

Introduction

Food allergy is developed to mediate the specific immunoglobulin (Ig) type E antibodies for specific proteins, termed allergens (1). Most food allergens have been reported as acid-stable (2) and resistant to enzymatic digestion in the gastric fluid (3, 4, 5). The resistant allergens penetrate the normal gastrointestinal tract (6). These antigenic proteins and peptides that traverse the mucosal endothelial barrier elicit an immune response that leads to an active secretion of the antigen-specific antibodies into the gut (7). Clinical doctors suggest that a sensitization to food allergens may occur in the gastrointestinal tract after food digestion (1, 7, 8). Food allergy, which is induced in all digestive systems, is especially evident in the intestine with abdominal pain, vomiting and/or diarrhea. If allergens enter the circulation system, they could be induced by systemic anaphylaxis (5, 9).

Ovalbumin (*Gal d* 2, OVA), a major allergen of egg, was reported to have resistance to enzymatic digestion and induce the gastrointestinal disease (3, 4). Meanwhile, several studies reported that the allergenicity of gamma-irradiated OVA was reduced due to the change of the conformation of the proteins (10-13). Also, Jeon et al. (14) reported that gamma-irradiated OVA showed a negative skin reaction in the skin prick test and that its binding capacity was reduced to 1/80 that of IgE-. Practically, however, such irradiated food materials have not been evaluated for their safety to allergenic individuals when eaten in food products.

Therefore, this study was conducted to evaluate the binding abilities of the rabbit IgG and egg-allergic patient IgE on gamma-irradiated OVA during proteolytic digestion.

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Materials and Methods

Production of the polyclonal anti-OVA IgG antibody and separation of IgG Polyclonal anti-OVA IgG was prepared as described in an earlier work (15), including the production and separation steps of the antibody.

Human sera Human sera were obtained from 28 patients less than 3 years of age (18 boys and 10 girls) diagnosed with IgE-mediated egg allergy. All the patients showed elevated egg specific IgE (0.8-100 KU/L) measured by AlaSTAT RIA (DPC Co., Los Angeles, CA, USA) or a Pharmacia CAP System FEIA (Pharmacia & Upjohn Diagnostics, Uppsala, Sweden). The pooled human sera were used for measuring the allergenicity of the irradiated OVA during the digestive reaction.

Gamma irradiation Isolated OVA was prepared (10 mg/mL) in 0.01 M phosphate buffered saline (PBS) and irradiated by a cobalt-60 irradiator (IR-79, Nordion International Ltd., Ontario, Canada) at an absorbed dose rate of 10 kGy. Dosimetry was performed with 5-mm-diameter alanine dosimeters (Bruker Instruments, Rheinstetten, Germany), and the free-radical signal was measured with a Bruker EMS 104 EPR analyzer. The irradiated sample was stored at 4°C before use.

Enzymatic digestion Peptic and tryptic digestion of OVA was carried out according to the modified method of Kovacs-Nolan et al. (16). Briefly, the pH of the prepared OVA (10 mg/mL) was adjusted to 2.0 with 0.1 M HCl. The ratio of pepsin (Sigma Chemical Co., St. Louis, MO, 2,790 units/mg, 1 mg/mL in 0.01 M HCl) to OVA was

1:200 (w/w). After 30 min incubation at 37°C, the pH of the solution was adjusted to between 7 and 8 with 0.1 M NaOH (pH 12.8) to inactivate the pepsin while allowing continued digestion by trypsin. The ratio of trypsin (Sigma Chemical Co., St. Louis, MO, 9,300 units/mg, 1 mg/mL in 0.01 M ammonium bicarbonate, pH 8.0) to OVA was 1:500 (w/w). Digestion was carried out at 37°C for 5, 10, 20, 30, 60, and 120 min, followed the addition of a trypsin inhibitor with the same amount of trypsin. The digested solutions were stored at 4°C for later experiment.

Competitive indirect-ELISA To measure the changes of the binding abilities of the polyclonal IgG and egg-allergic patient IgE to the gamma-irradiated and enzyme-treated OVA, the binding abilities were monitored by a competitive indirect-ELISA (Ci-ELISA) individually formatted with Abs. Briefly, polystyrene, flat-bottom, microtiter plates (Maxisorp, Nunc, Kamstrup, Denmark) were coated with 100 μl of each intact OVA (10 μg/mL in 0.2 M bicarbonate buffer, pH 9.6), and incubated overnight at 4°C. All subsequent steps were carried out at 37°C for 2 h and the plates were washed three times between each step with PBS containing 0.05% (v/v) Tween 20. To reduce the nonspecific binding, the plates were blocked with 120 μl of PBS containing 1% (w/v) bovine serum albumin (BSA). Fifty microliter of a standard solution or sample solution was added to the wells, and then 50 μl of each antibody solution (polyclonal IgG, 1:3,000; egg-allergic patient IgE, 1:20) was added and incubated. After the addition of 100 μl of a tracer solution to the wells, 100 μl of *o*-phenylenediamine (Sigma Chemical Co., St. Louis, MO) in a 0.1 M phosphate-citrate buffer (pH 5.0) with 0.04% (v/v) hydrogen peroxide (35% H_2O_2) was added to the well for a color reaction, and allowed to incubate for 30 min. The reaction was stopped with 50 μl of 2.0 M H_2SO_4 without a washing step. Absorbance was measured at 492 nm with an ELISA reader (CERES UV-900C, BIO-TEK instruments Inc., MI, USA).

Statistical analysis All the experiments were repeated 5 times with observation performed three times. The means were used to evaluate the differences of the concentrations of the allergens from the Ci-ELISA results. These data were analyzed by general linear procedures, least square means, and Duncan's multiple range test. SAS® software (17) was used for the statistical analysis.

Results and Discussion

The measurement results for the binding abilities of the enzyme-treated OVA differed between antibodies. Table 1 shows the changes of binding ability of the polyclonal rabbit anti-OVA IgG on intact and irradiated OVA. Irradiated OVA was detected at a level more than two times higher than that of intact OVA by IgG before enzymatic treatment. A similar result was previously reported for the inner epitopes in OVA being exposed to the surface due to the conformational alteration of OVA by gamma irradiation (10-12). The binding abilities of IgG on the intact and irradiated OVA quantitatively decreased after peptic digestion followed by trypsin treatment, whereas the binding abilities of both increased after a few

Table 1. Changes of the concentration of gamma-irradiated and enzyme-treated ovalbumin detected by Ci-ELISA formatted with polyclonal rabbit anti-OVA IgG[1)]

Irradiation dose	Concentration (μg/mL) of OVA after enzymatic treatment[2)]					
	Non-treated	Pepsin	Trypsin			
		30[3)]	5	30	60	120
0 kGy	14.43[a]	4.89[b]	0.05[d]	0.11[d]	0.06[d]	2.50[c]
10 kGy	35.82[a]	9.24[b]	0.51[c]	0.40[c]	0.32[c]	12.80[b]

[1)]The competitive concentration of the irradiated and enzyme-treated ovalbumin was 12.50 μg/mL.
[2)]Different letters within a row indicate significant differences ($p<0.05$).
[3)]Indicates time (min) of hydrolysis with pepsin and trypsin at 37°C. Trypsin was added with pepsin to the digested OVA.

Table 2. Changes of the concentration of gamma irradiated and enzyme-treated ovalbumin detected by Ci-ELISA formatted with egg-allergic patient IgE[1)]

Irradiation dose	Concentration (μg/mL) of OVA after enzymatic treatment[2)]					
	Non-treated	Pepsin	Trypsin			
		30[3)]	5	30	60	120
0 kGy	17.92[a]	7.89[b]	1.39[c]	1.06[c]	7.70[b]	17.49[a]
10 kGy	0.24[a]	0.28[a]	0.12[b]	0.19[a]	0.15[b]	0.25[a]

[1)]The competitive concentration of the irradiated and enzyme-treated ovalbumin was 12.50 μg/mL.
[2)]Different letters within a row indicate significant differences ($p<0.05$).
[3)]Indicates time (min) of hydrolysis with pepsin and trypsin at 37°C. Trypsin was added with pepsin to the digested OVA.

minutes of the trypsin treatment. However, because a similar tendency was shown in both the intact and irradiated OVAs, regardless of the measured concentration in polyclonal IgG-response, the irradiated OVA seems to have been digested as intact OVA.

For the egg-allergic patient IgE reacted with enzyme-treated OVA, the binding capacities differed between the non-irradiated and irradiated OVA (Table 2). Patient IgE reduced its binding ability to the intact OVA for up to 30 min after a trypsin treatment, but it increased thereafter. This pattern demonstrated a particular response of the allergic patient IgE. Most food hypersensitivities induce acute symptoms within a period from several minutes to two hours, especially if the response occurs in the intestine (7, 8). We could demonstrate such a food-allergic response by the increased IgE-binding ability for native OVA in the intestinal condition. It is considered that the epitopes within the OVA molecule are exposed more by the proteolysis of the pepsin and trypsin. Meanwhile, the concentration of the irradiated OVA detected by IgE suddenly reduced after irradiation (12, 15). The initial low concentration of the irradiated OVA, which had been reduced by irradiation, was stably maintained during the enzymatic treatment. It was shown that the epitopes of the irradiated OVA were not exposed by pepsin or trypsin. Kume and Matsuda (10) reported that gamma irradiation could change the conformation-dependent antigenic structures without any alteration of the sequential epitopes. The results of the binding abilities of the polyclonal IgG and egg-allergic patient IgE indicate that the concentration of the irradiated OVA detected by IgE was affected by the gamma irradiation, but not by the enzymatic treatment. It

was considered that irradiation may affect both the primary and tertiary structures to a certain degree.

In the development of a new method to reduce or inhibit a food allergy, the evaluation of the allergenic safety in a digestion system is very important. The results presented here show the potential application of gamma irradiation for the reduction of the food allergenic responses caused by OVA.

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References

1. Sampson HA. Food allergy. Food allergy Part 1: immunopathogenesis and clinical disorders. J. Allergy Clin. Immunol. 103: 717-728 (1999)
2. Hefle SL. The chemistry and biology of food allergens. Food Technol. March pp. 86-89 (1996)
3. Astwood JD, Leach JN, Ruchs RL. Stability of food allergens to digestion in vitro. Nat. Biotechnol. 14: 1269-1273 (1996)
4. Fu TJ, Abbott UR, Hatzos C. Digestibility of food allergens and nonallergic proteins in simulated gastric fluid and simulated intestinal fluid- a comparative study. J. Agric. Food Chem. 50: 7154-7160 (2002)
5. Metcalfe DD. The nature and mechanism of food allergies and related diseases. Food Technol. May pp. 136-139 (1992)
6. Shao L, Serrano D, Mayer L. The role of epithelial cells in immune regulation in the gut. Semi. Immunol. 13: 163-175 (2001)
7. Murphy S, Walker WA. Antigen absorption. pp. 93-106. In: Food allergy: Adverse reactions to foods and food additives. Blackwell Science, USA (1997)
8. Sampson HA. 9. Food allergy. J. Allergy Clin. Immunol. 111: S540-547 (2003)
9. Hayakawa K, Kinko Y, Linko P. Mechanism and control of food allergy. Lebensm-Wiss. u-. Technol. 32: 1-11 (1999)
10. Kume T, Matsuda T. Change in structural and antigenic properties of proteins by radiation. Radiat. Phys. Chem. 46: 225-231 (1995)
11. Lee JW, Yook HS, Cho KH, Kim CJ, Byun MW. Effects of heat treatment on the antigenicity and gamma-irradiated egg white albumin. J. Korean Soc. Food Sci. Nutr. 30: 848-853 (2001a)
12. Lee JW, Yook HS, Cho KH, Lee SY, Byun MW. The changes of allergenic and antigenic properties of egg white albumin (Gal d 1) by gamma irradiation. J. Korean Soc. Food Sci. Nutr. 30: 500-504 (2001b)
13. Masuda T, Koseki SY, Yasumoto K, Kitabatake N. Characterization of anti-irradiation-denatured ovalbumin monoclonal antibodies. Immunochemical and structural analysis of irradiation-denatured ovalbumin. J. Agric. Food Chem. 48: 2670-2674 (2000)
14. Jeon GR, Lee JW, Byun MW, Lee SY. Reduced allergenicities of irradiated egg white ovalbumin determined by skin prick test and ELISA inhibition test. J. Asthma Allergy Clin. Immunol. 22: 711-719 (2002)
15. Seo JH, Lee JW, Lee YS, Lee SY, Kim MR, Yook HS, Byun MW. Change of an egg allergen in a white layer cake containing gamma-irradiated egg white. J. Food Prot. 67: 1463-1468 (2004)
16. Kovacs-Nolan J, Zhang JW, Shigeru H, Mine Y. Immunochemical and structural analysis of pepsin-digested egg white ovomucoid. J. Agric. Food Chem. 48: 6261-6266 (2000)
17. SAS. SAT/STAT: User's Guide. SAS institute Inc., Cary, NC, USA (1998)