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Changes of Allergenicity and Conformational Structure of Egg Ovomucoid by Gamma Irradiation in the Basic Condition

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Abstract

This study was conducted to evaluate the possibility of gamma radiation for reducing egg allergies through the observation of conformational and allergenic changes of egg ovomucoid (OM) in basic pH conditions. An OM solution of 2.0 mg/mL was individually prepared with different pH conditions, pH 7.0, 9.0 or 10.0, and was irradiated with the absorbed dose of 10 kGy. Irradiated OM solutions were tested by Ci-ELISA formatted with egg-hypersensitive patients' IgE. Binding abilities of IgE to OM in irradiated solution decreased with the increase of pH. Turbidity of the solution highly increased by irradiation and the increase of pH. A yellowish color was observed in the irradiated OM solution of basic condition. Coagulation of OM by irradiation decreased with the increase of pH, when observed by SDS-PAGE.

Key words: ovomucoid, gamma irradiation, basic condition, and allergenicity

INTRODUCTION

Egg is one of the most allergenic foods, and minute amounts of egg can result in symptoms within minutes, including life-threatening anaphylaxis (1). Egg white appears to be more allergenic than egg yolk. Among the egg white proteins, ovalbumin and ovomucoid (OM) have been recognized as major allergens (2). OM is a glycoprotein with molecular weight of 28 kDa, consisting of three well-separated domains. OM is relatively stable when heated to 100°C in the acidic and neutral pH condition ($pH \leq 7$), however, it is easily denatured by heating below 80°C in basic condition ($pH \geq 9$) (3). Mine and Zhang (4), and Besler et al. (5) found that there was significantly more patient's IgE binding activity to the third domain of OM and carbohydrate moieties did not have an affect on its allergenicity. For reducing OM-induced hypersensitivity, destruction of linear (sequential) epitopes on the third domain is very important.

The structural modification of food allergens by gamma irradiation was recently observed in several experiments and some results at present have indicated that ionizing radiation could reduce allergenicity or antigenicity by the destruction and/or denaturation of binding epitopes of food proteins (6-9). Unfortunately, however, authors observed that antigenicity of OM was not easily changed by gamma irradiation (10). Therefore, authors hypothesize that the gamma irradiation at a high pH may change the allergenicity of OM.

This study was conducted to evaluate the usability of gamma radiation for reducing egg allergies through the observation of conformational and allergenic changes of OM in the basic pH condition.

MATERIALS AND METHODS

Protein and antibodies

Isolated egg OM was purchased from Sigma Chemical Co. (St Louis, MO, USA) to use standard allergen of egg white protein. Human sera subjected in the previous study (9) were also used in this study. Horseradish peroxidase (HRP) conjugated rabbit anti-human IgE IgG was purchased from Sigma Chemical Co. and used as secondary antibody with chromogenic enzyme.

Sample preparation and treatment

OM was individually dissolved in 0.01 M sodium phosphate buffered saline (PBS) with NaCl of 0.15 M, pH 7.4, 9.0 and 10.0, and adjusted to a final concentration of 2.0 mg/mL with the buffers of the same pH. The solution (30 mL) was put into a glass tube (φ 1.0 cm, glass thickness 1 mm) with a cap and irradiated.

Gamma irradiation was carried out to obtain total absorbed dose of 10 kGy at 10±0.5°C by a cobalt-60 irradiator (IR-79, Nordion International Ltd., Ontario, Canada) of 3.7 PBq (100 kCi) activity. The absorbed dose was

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determined by Fricke dosimetry (11).

For recovering pH of the basic solution (pH 10.0) to 7.4, a half portion (15 mL) from the irradiated solution was put into a dialysis tube (pore size, <12,600 dalton, Spectrum® Medical Industries, Inc., Houston TX, USA) and dialyzed in 2 L of 0.01 M PBS of pH 7.4 overnight at 4°C with change of buffer twice.

The protein concentration of sample solutions was determined by using a bicicinchonic acid protein assay kit (Sigma Chemical Co., St Louis, MO, USA) with bovine serum albumin (BSA) solution as a standard at 562 nm with a spectrophotometer (UV-1600PC, Shimadzu Corp., Kyoto, Japan) by the method described previously (12).

Competitive indirect ELISA

Competitive indirect ELISA (Ci-ELISA) was formatted with the patients' IgE for evaluating binding abilities of IgE to samples by the method reported previously (13). Briefly, polystyrene flat-bottom microtiter plates (Maxisorp, Nunc, Kamstrup, Denmark) were coated with 100 μL of intact OM solution (10.0 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 three times with PBS containing 0.05% (v/v) tween 20 (PBST). To reduce the non-specific binding, the plates were blocked by incubation for 1.5 h with 120 μL of PBS containing 1% (w/v) BSA. After washing, 50 μL of sample solution, which was serially diluted with PBS of same pH in the concentration from 500 to 0.12 g/mL, was added to 4 coated and blocked wells, and then 50 μL of IgE solution diluted 25 times in PBS was added. The plates were incubated for 2 h, and then washed three times with PBST. After the addition of 100 μL of the secondary antibody solution to the wells, the plates were incubated for 1.5 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 for color reaction for 20 min before stopping it with 2.0 M H_2SO_4 (50 μL/well). The absorbance was measured at 492 nm by an ELISA reader (CERES UV-900C, BIO-TEK instruments Inc., New York, NY.).

Turbidity and Hunter's color values

To observe the aggregation of OM in solution induced by irradiation, the turbidity of the sample solution was determined by the method reported previously (14) based on measurement of absorbance at 280, 320, 340 and 660 nm with a spectrophotometer (UV-1600PC, Shimadzu Corp., Kyoto, Japan).

A sample solution of 10 mL was transferred into a glass cell (CM-A97, optical path length, 2 mm), placed on the cell holder and measurement was performed using the Color Difference Meter (Spectrophotometer CM-3500d, Minolta Co., Ltd. Osaka, Japan) with an illuminant D65/10° observer for measuring the changes of the color values, especially b-value. A large size aperture was used in triplicate measurements. The Hunter color L-, a-, and b-values were obtained by using Spectra Magic software (version 2.11, Minolta Cyberchrom Inc. Osaka, Japan).

SDS-PAGE

SDS-PAGE (5~15% gradient gel) for sample solutions (2 mg/mL) was performed by Laemmli's method (15) using a Hoefer vertical electrophoresis apparatus SE-260 (Pharmacia Biotech., Uppsala, Sweden), and a staining and destaining method was done as described previously (16). A molecular weight marker was purchased from Bio-Rad Laboratories (Hercules, CA, USA) to determine the molecular weights.

Statistical analysis

All samples were prepared and treated in triplicate and the experiments were repeated 5 times. The means and standard deviations were used to evaluate the slope rates of calibration curves, and turbidity and Hunter's color values. The data were analyzed by general linear procedures, least square means, and Duncan's multiple range test. SAS® software (17) was used to achieve the above-mentioned analysis.

RESULTS AND DISCUSSION

Changes of binding ability of IgE

Calibration curves of egg hypersensitive patients' IgE against OM in sample solutions irradiated under different pH conditions were obtained for the comparison of the binding ability of IgE (Fig. 1). The increase of pH decreased the binding ability of IgE more easily than the neutral condition, when OM was irradiated. The change of binding ability can be determined by the movement of the curve. In general a reversed semilogarithmical curve is obtained in ELISA formatted with antigen-coating method as this study (10,13,18) and the reactivity of antibody against antigen in a sample can be evaluated by the observation of the movement of the curve (8). If the curve of a sample moved to the left side, compared with a standard curve (control), antibody was a more recognized antigen in the sample than that in control. In contrary, if the curve moved to the right side, antibody was a less recognized antigen in the sample than that in control. The curves moved to the right side by the increase of pH, and the movement to the right side also indicated that the IgE did not well recognize epitopes on antigen.

The result showed that gamma irradiation in high pH conditions might cause more changes of the epitopes

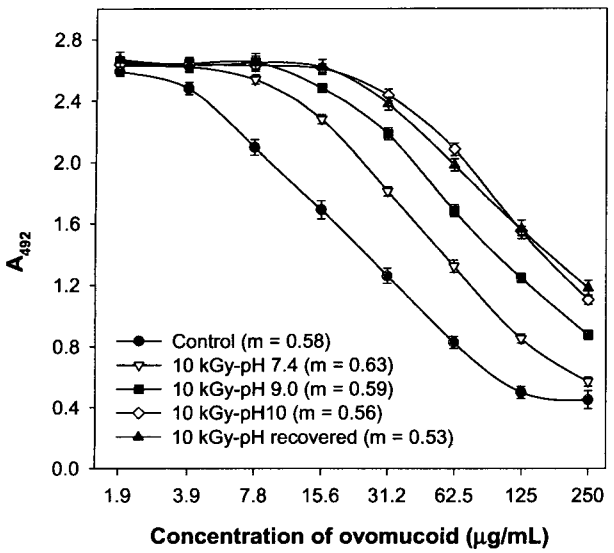

Fig. 1. Calibration curves of egg white hypersensitive patients' IgE against ovomucoid 10 kGy gamma-irradiated under different pH conditions by Ci-ELISA. Sample solution (2.0 mg/mL) was individually prepared in each pH, irradiated, serially diluted and then tested. m is the slope of the curve obtained from the binding rate of IgE to coated intact OM.

on OM than in neutral conditions. The number of epitopes decreased by the denaturation of the molecule such as fragmentation or conformational release of the molecule, or aggregation among the molecules, etc, caused by gamma irradiation (19,20). When pH 10.0 was recovered to the physiological condition (pH 7.4) after irradiation, the allergenicity of OM did not revert to the irradiated state at pH 7.4 and maintained a decreased state at pH 10.0. This result suggested that conformational IgE epitopes which were the paring of structurally adjacent amino acids in domain I and II, or domain II and III in native OM (3,4) were cleaved or destructed in basic condition and were not recovered; that is, the denaturation of OM in the basic condition was irreversible and the adjustment of pH for food processing including egg whites with reduced allergenicity might be adequately used to match the property of products.

The slopes of the calibration curves were not different. The result showed that OM was structurally changed with similar allergenicity, and that the epitopes on the protein were masked by aggregation of the molecule (21). The slopes of the curves obtained by competition between coated standard OM and irradiated proteins offered important clues regarding conformational changes that may occur in the epitopes following irradiation in the high pH condition (8,9). Increase of the slope indicates the reduction of binding ability of antibody to irradiated protein. If the slopes were similar, then the epitopes were not likely to be altered in the structure. However, the number of the epitopes appeared to change suggesting alterations in solubility or aggregation that might mask some epitopes (22). The result suggested that the epitopes were not being altered.

The binding ability of a patient's IgE to domain III was higher than to other domains in OM molecule, and the amino acids within the IgE binding epitope of domain III was important for the allergenic reaction of OM (3,4). Irradiation in the basic condition might not directly destruct the linear IgE epitope on domain III and might decrease the number exposed on the surface of OM by the coverage generated by aggregation among the molecules.

Turbidity and Hunter's color values

Turbidity of the sample solution slightly increased by the increase of pH and the increase was highly raised by irradiation (Table 1). Because OM is structurally unstable in its basic condition (pH≥9) (3), the increase by radiation might be more easily generated in a high pH than in 7.4. The result showed that the structure of protein was released and coagulated by radiation. Protein-protein interaction and increase of hydrophobicity by ionizing radiation cause the turbid of OM solution to increase, and the increase can be also observed in other reports (6,13,19). Protein-protein interaction is generated by active aldehyde (ketone) group produced by destruction of bond between chiral carbon and amino group in amino acids (23) and increase of hydrophobicity is caused by the release of the third structure of protein due to reduction of disulfide bone and destruction of hydrogen bond between subunits (6,7).

A yellowish color was visually observed in irradiated solutions (Table 2). When color values were determined by a color difference meter, the significant increase of b-value was observed in irradiated samples. The increase of pH caused the increase of b-value in only irradiated samples and the increase was not observed in non-irradiated samples. L- and a-values slightly decreased in irradiated samples and the decrease was gone with the

Table 1. Changes of OD values of ovomucoid solution (2.0 mg/mL) gamma-irradiated at different pH conditions

Irradiation dose (kGy)	PH	Wavelength (nm)			
		280	320	340	660
0	7.4	0.84[a]	0.09[a]	0.074[a]	0.001
	9.0	0.94[b]	0.102[b]	0.087[b]	0.001
	10.0	0.95[b]	0.109[c]	0.092[c]	0.001
	SEM	0.012	0.0008	0.0008	0.0006
10	7.4	2.26[a]	0.67[a]	0.39[a]	0.002[a]
	9.0	2.68[b]	0.82[b]	0.46[b]	0.004[b]
	10.0	2.91[c]	1.05[c]	0.60[c]	0.004[b]
	SEM	0.053	0.014	0.009	0.0008

[a-c]Different letters within a column with the same dose differ significantly ($p<0.05$).

Table 2. Hunter color L-, a-, and b-values of irradiated ovomucoid dissolved in PBS buffer at different pHs

Color	Irradiation dose (kGy)	pH of buffer			SEM
		7.4	9	10	
L	0	102.42^{x}	102.41^{x}	102.43^{x}	0.008
	10	102.32^{ay}	102.28^{aby}	102.25^{by}	0.014
	SEM	0.017	0.010	0.014	
a	0	-0.0003^{x}	-0.0003^{x}	-0.0004^{x}	0.01
	10	-0.17^{az}	-0.19^{by}	-0.24^{cy}	0.008
	SEM	0.003	0.007	0.009	
b	0	-0.10^{x}	-0.10^{x}	-0.11^{x}	0.01
	10	0.58^{cy}	0.68^{by}	0.87^{ay}	0.006
	SEM	0.004	0.006	0.006	

[a-c]Different letters within the same row differ significantly ($p<0.05$).
[x,y]Different letters within a column with the same color value differ significantly ($p<0.05$).

increase in pH. Though statistical differences were appeared at L- and a-values, the apparent change of color in OM solution was caused by the changes to the yellowish color. Unfortunately, the changes of the color of OM solution by irradiation have not been reported. Further research is needed to elucidate the changes of the color.

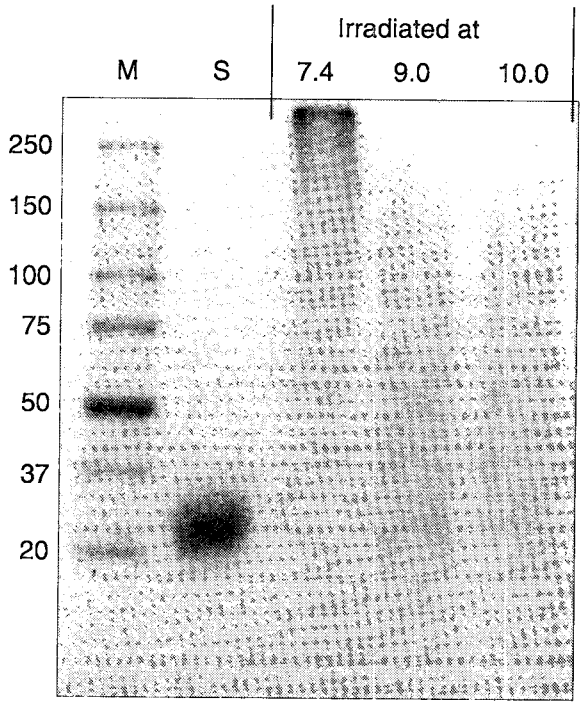

Fig. 2. SDS-PAGE of ovomucoid in solutions 10 kGy gamma irradiated in different pHs. M, molecular weight standard marker; S, intact ovomucoid in pH 7.4; 7.4, ovomucoid irradiated at pH 7.4, 9.0, ovomucoid irradiated at pH 9.0; 10.0, ovomucoid irradiated at pH 10.0. Numbers on the left side indicate the molecular weights of the standard marker.

SDS-PAGE

Electrophoretogram of OM solution irradiated in the basic condition is shown in Fig. 2. The band of about 28 kDa disappeared in irradiated samples. Trace spread from the starting line of separating gel was observed in the sample irradiated at pH 7.4, and traces were down from the start line to below 150 kDa in pH 9.0 and 10.0. By gamma irradiation, a protein molecule can be broken down to smaller molecules or coagulated to larger molecules by interaction among the molecule (6,19,22). OM was coagulated and became very large aggregates by gamma irradiation in the presence of oxygen. Increase in pH might induce smaller aggregates with large molecular weight ranges to be generated by the hydroxyl group in the basic condition, instead of generation of the new big molecular weight in neutral condition.

In conclusion, data obtained from this study presented basic information that the irradiation processing available to reduce egg allergies can be used for treating raw materials for processed food.

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