Changes in Allergenicity of Porcine Serum Albumin by Gamma Irradiation

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Abstract

Pork is an excellent source of essential nutrients such as protein. However, pork can trigger hypersensitivity and serum albumin of pork is known as major allergen. In this study, to evaluate the effect of gamma irradiation on the allergenicity of porcine serum albumin (PSA), PSA solution was irradiated at 3, 5, 7, 10, 15, and 20 kGy. The changes in the ability of PSA to bind IgG and patient’s serum caused by gamma irradiation were observed by ci-ELISA and immunoblotting. SDS-PAGE was used for measuring the conformational change of gamma-irradiated PSA. The ability of 3-kGy-irradiated PSA to bind p-IgG and patient’s serum was decreased to 30% and 15%, respectively. The binding ability showed no significant differences among all irradiated samples. SDS-PAGE showed that the irradiated PSA bands were degraded and aggregated. Immunoblotting of irradiated PSA revealed that IgG and patient’s serum were rarely recognized at 3 kGy. Therefore, gamma irradiation could be applied to less-allergenic pork products.

Key words: porcine serum albumin, allergenicity, gamma irradiation

Introduction

Studies of meat allergy are rare compared to common food allergies such as cow’s milk, shrimp, and peanuts (Fiocchi et al., 1998; Kanny et al., 1998; Kim et al., 2008) because meat is less allergenic than common allergy-inducing foods. But, meat and its products are important foods because it is rich in protein and participates in the development of children’s gastrointestinal mucosa. Thus, studies of meat allergy have become crucial for allergic subjects.

Meat allergy has been mostly studied in children in association with cow’s milk and beef allergy (Fiocchi et al., 1995; Han, 2006). Although pork allergy is relatively rare and has not been studied well, the symptoms of pork allergy manifest as atopic dermatitis and oral allergy syndrome (OAS) (Johansson et al., 2001). The prevalence of pork allergy was found to be 58% among 57 subjects with suspected meat allergy in USA (Ayuso et al., 1999) and 3% based on questionnaire study in Japan (Ikura et al., 1999). Also, the frequency of sensitization in the skin prick test (SPT) to pork was shown to be 2% in Germany (Bohler et al., 2001). In Korea, incidence of pork allergy showed 91.2% at 0-3 ages groups and 54.2% at 4-6 ages groups as a results of IgE tests (Moon et al., 2007). Serum albumins are well known as the most important meat allergen and are found in protein of milk (Wahn et al., 1981; Restani et al., 1995) and pet hair/dander (Spitzauer et al., 1997; Spitzauer et al., 1995). Most allergen of pork is serum albumin (PSA) and besides serum albumin, several other IgE-binding proteins (60, 50 and 44 kDa) have been detected in pork (Wang et al., 2002; Latzer et al., 1998).

Irradiation technique is known to enhance shelf-life and/or improve microbiological safety of raw and processed food materials without damaging nutritional quality (WHO, 1999). Also, effects of gamma irradiation on allergenicity or antigenicity of food allergens have been evaluated in recent years. Some papers have indicated
that gamma irradiation could alter conformational structure of antigen by radicals generated from radiolysis of water (Byun et al., 2000; Kume and Matsuda, 1995). For instance, Byun et al. (2000) reported that amount of shrimp heat stable protein (HSP) showed a decrease and that its antigenicity decreased in a dose-dependent manner as HSP was broken down and coagulated by gamma irradiation. Presently, in our country, few studies are reported to reduce the allergenicity of PSA, pork major allergen. Therefore, the aim of this study was to investigate changes in allergenicity and structure of PSA by gamma irradiation.

Materials and Methods

Antigen and antibody

Antigen, porcine serum albumin (PSA), was purchased from Sigma (Sigma Chemical Co., St Louis, MO, USA). Goat polyclonal-IgG was obtained from Bethyl (Bethyl laboratories Inc., Texas, USA). Serum of allergic patient to pork was obtained from Yonsei University College of Medicine. Anti-goat IgG peroxidase conjugate was purchased from Sigma (Sigma Chemical Co., St Louis, MO, USA).

Ci-ELISA (competitive indirect-enzyme linked immunosorbent analysis)

The modified ci-ELISA method of Lee et al. (1998a) was conducted to investigate the allergenicity of physically treated PSA. Ten µg/mL PSA in 0.2 M bicarbonate buffer (pH 9.6) was coated into the wells (Nunc, Kamstrupvej, Denmark) at 4°C, overnight and blocked for 2 h at 37°C with 1% gelatin in 0.01 M PBS (phosphate buffered saline, pH 7.3). Fifty microliters of non- and physically treated PSA solution (12 µg/mL) and 50 µL of diluted p-IgG (1:500)/patient’s serum (1:32) in 0.01 M PBS (pH 7.3) were added into the wells and incubated for 2 h at 37°C. One hundred microliters of diluted secondary antibody (anti-goat IgG-1:40,000, anti-human IgE-1:250) were added into the wells and incubated for 2 h at 37°C. One hundred microliters of phosphate citrate buffer (pH 5) containing 0.05% O-phenylenediamine (Sigma Chemical Co.) and 0.04% H2O2 was added to the wells and incubated for 30 min at 37°C and stopped by 50 µL of 2 M H2SO4. Absorbance was measured at 490 nm using the ELISA reader (Model 550, Bio-rad, USA). For 100% binding between coated antigen and primary antibody, 50 µL of each primary antibody and 0.01 M PBS (pH 7.3) were added to the well. One hundred microliters of 0.01 M PBS (pH 7.3) was used as the blank. After incubation, each step was washed three times in 0.01 M PBST (0.01 M PBS containing 0.05% (v/v) Tween 20). The binding ability of physically treated PSA to p-IgG and patient’s serum was calculated as below.

\[
\text{Binding ability (\%)} = \frac{\text{Absorbance of physically treated PSA}}{\text{Absorbance of untreated PSA}} \times 100
\]

Titration curve of ci-ELISA

To evaluate the degree of maximum binding between coated antigen and antibody, the modified titration curve method of Lee et al. (1998b) was carried out. Each PSA dilution (5, 10, and 20 µg/mL) in 0.2 M bicarbonate buffer (pH 9.6) was coated into the well at 4°C, overnight and blocked in 0.01 M PBS (pH 7.3) containing 1% gelatin for 2 h at 37°C. One hundred microliters of diluted p-IgG (2 to 0.0156 µg/mL) was added to the wells and incubated for 2 h at 37°C. All subsequent procedures were the same as the method of the ci-ELISA described.

Standard curve of ci-ELISA

The well was coated with 10 µg/mL of PSA solution in 0.2 M bicarbonate buffer (pH 9.6) at 4°C, overnight. The well was then blocked with 1% gelatin in 0.01 M PBS (pH 7.3) for 2 h at 37°C. Fifty microliters of diluted secondary antibody (anti-goat IgG-1:40,000, anti-human IgE-1:250) were added into the wells and incubated for 2 h at 37°C. One hundred microliters of phosphate citrate buffer (pH 5) containing 0.05% O-phenylenediamine (Sigma Chemical Co.) and 0.04% H2O2 was added to the wells and incubated for 30 min at 37°C and stopped by 50 µL of 2 M H2SO4. Absorbance was measured at 490 nm using the ELISA reader (Model 550, Bio-rad, USA). For 100% binding between coated antigen and primary antibody, 50 µL of each primary antibody and 0.01 M PBS (pH 7.3) were added to the well. One hundred microliters of 0.01 M PBS (pH 7.3) was used as the blank. After incubation, each step was washed three times in 0.01 M PBST (0.01 M PBS containing 0.05% (v/v) Tween 20). The binding ability of physically treated PSA to p-IgG and patient’s serum was calculated as below.

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\]

Gamma irradiation treatment

The PSA solution was transferred into a cobalt-60 irradiator (IR-79, Nordion International Ltd., Ontario, Canada) with 100-KiloCurie activity at 10±0.5°C and irradiated at a dose rate of 10 kGy/h. The applied dose levels were 3, 5, 7, 10, 15, and 20 kGy.

SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis)

SDS-PAGE was carried out by the method of Laemmli (1970) to determine the degree of PSA decomposition by gamma irradiation. Twelve µg/mL of PSA solution was used as the SDS-PAGE sample. SDS-PAGE was performed with a 12% running gel and 4.5% stacking gel. The gels were stained with CBB (coomassie brilliant blue R250) solution and destained with 5% methanol and 7% acetic acid solution. Molecular weight (Mw) markers
were purchased from BioLabs (P7702S, New England BioLabs, Beverly, Massachusetts, USA). The Mw standards were insulin A and B chains (2.3 kDa and 3.4 kDa, respectively), aprotinin (6.5 kDa), lysozyme (14 kDa), trypsin inhibitor (20 kDa), triosephosphate isomerase (26 kDa), lactate dehydrogenase (36 kDa), MBP (42 kDa), glutamic dehydrogenase (55 kDa), serum albumin (66 kDa), phosphorylase b (97 kDa), β-galactosidase (116 kDa), MBP-β-galactosidase (158 kDa), and myosin (212 kDa). A scanner (Power Look III, Amersham Pharmacia Biotech Company, Piscataway, New Jersey, USA) was used to analyze the gel.

**Immunoblotting**

After electrophoresis, electrotransfer of separated polypeptides onto methanol-activated polyvinylidene difluoride (PVDF) membrane was carried out by the method of Towbin et al. (1979). The protein was transferred for 5 h at 150 mA using a buffer containing 0.25 M Tris-base, 1.92 M Glycine, and 20% Methanol. After transfer, the protein band was identified by 0.1% (w/v) Ponceau-S and 5% acetic acid and destained with 7% acetic acid. Each strip was blocked with 3% gelatin solution (3% gelatin, 0.2 M Tris-HCl buffer (pH 7.5), 1.5 M NaCl) for 1 h and incubated with diluted p-IgG (1:500)/patient’s serum (1:30) in 1% gelatin solution (1% gelatin, 0.2 M Tris-HCl buffer (pH 7.5), 1.5 M NaCl) for 3.5 h. The strip was incubated with diluted anti-goat/human IgG/IgE (1:1000) in TBST (Tris-buffered saline containing 0.1% (v/v) tween 20) for 1 h. The protein was visualized with DAB solution (3,3’-diaminobenzidine, Sigma Co.) and rinsed with deionized water. The blots were scanned on a scanner (Power Look III, USA). Except for the blocking step, all steps were washed out three times with TBST.

**Statistical analysis**

Data were analyzed by ANOVA for significance using SAS software (SAS Institute Inc., Cary, NC, USA). Mean comparisons were done with Duncan’s multiple range test \( (p<0.05) \) to determine significant differences between sample means.

**Results and Discussion**

**Standard curve**

The concentration of PSA in the sample solutions to p-IgG was calculated using the equation when the standard curve was obtained using 10 µg/mL of antigen and 2 µg/mL of p-IgG. And the concentration of PSA in the sample solutions to serum from allergic patient to pork was calculated using the equation when the standard curve was obtained with 10 µg/mL of antigen and 1:32 dilution of pig-allergic patient’s serum.

\[
x = e^y
\]

\[
x = \text{concentration of PSA to goat p-IgG}
\]

\[
y = \text{absorbance value}
\]

\[
x = \text{concentration of PSA to serum from allergic patient to pork}
\]

\[
y = \text{absorbance value}
\]

The PSA solution to p-IgG was quantitatively determined in the range 0.78 to 200 µg/mL (Fig. 1) and the PSA solution to serum from allergic patient to pork was quantitatively determined in the range 0.012 to 3.13 µg/mL (Fig. 2).

**SDS-PAGE and Immunoblotting patterns of gamma-irradiated PSA**

SDS-PAGE patterns of gamma-irradiated PSA at doses of 3 to 20 kGy are shown in Fig. 3. The gamma-irradiated PSA band (66 kDa) degraded into low-molecular-weight molecules and slightly aggregated to form high-molecular-weight molecules (lane 2 to 7) as compared with intact PSA band. At a dose of 20 kGy (lane 7), degraded fragments disappeared more in comparison with those at doses ranging from 3 (lane 2) to 15 kGy (lane 6). The structural change of food protein by radiation is induced...
by formation of radiolytic products/free radicals indirectly from water (Jeon et al., 2002). Those radicals are known to attack the aromatic and thiol site more than other residues in a protein (Lee and Byun, 2003). Irradiation can break protein molecule down to smaller molecules or aggregate to form larger molecules (Davies and Delsignore, 1987). This result corresponded to the changes of other irradiated proteins such as β-lactoglobulin (Lee et al., 2001), bovine and porcine plasma protein (Lee et al., 2003), and ovalbumin (Lee et al., 2007). These irradiated proteins were also broken down or coagulated by gamma irradiation treatment. Filail-Mouhim et al. (1997) reported that low-molecular-weight peptides occur by disulfide-bond and peptide-bond cleavage of protein by free radicals generated from gamma irradiation. Kume and Matsuda (1995) reported that production of molecules with high-molecular-weight by gamma irradiation is induced by exposure of hydrophobic sites. For testing reactivity against intact PSA and gamma-irradiated PSA, immunoblotting technique was used (Fig. 3). The gamma-irradiated PSA solution at 3 to 20 kGy did not recognize anti-PSA IgG and patient’s serum. These results indicated that PSA did not recognize the IgG and patient’s serum because of structural changes of PSA epitope by gamma irradiation.

### Changes in allergenicity of gamma-irradiated PSA

The changes in allergenicity to gamma-irradiated PSA solution (3, 5, 7, 10, 15, and 20 kGy) were investigated. Binding ability of PSA treated with gamma irradiation to p-IgG sharply was decreased to less than 30% when PSA solution was treated with a dose of 3 kGy (Fig. 4). The binding ability was about 25% at 15 kGy and 20 kGy, 27, 23, and 28% at 3, 5, and 7 kGy, respectively (Fig. 4). The ability of gamma-irradiated PSA solution (3, 10, and 20 kGy) to bind serum from allergic patient to pork was decreased more to 15, 11, and 19% as compared with p-IgG (Fig. 5). Both results (Fig. 4 and 5) showed no significant differences among irradiated samples. The gamma irradiation was proved to cause reduction in the number of epitopes by the denaturation of the protein such as fragmentation or conformational release of the molecule or aggregation among the molecules (Filail-Mouhim et al., 1997; Kume et al., 1994). In the case of bovine serum albumin (BSA), a similar result was obtained. Kume and Matsuda (1995) reported that reactivity of BSA to anti-BSA antibody by gamma radiation was diminished at 4...
kGy. They reported that the reduction in the antigenicity of BSA following gamma irradiation was caused by the destruction of secondary structure and the exposed the hydrophobic amino acids. Thus, our results indicated that gamma irradiation caused the reduction in allergenicity of PSA because of changes of IgG- and IgE-binding epitopes of PSA by modification of structure. Other food allergens have been also studied for reducing their allergenicity by gamma irradiation.; Lee et al. (2000) reported that major shrimp allergen, tropomyosin, was coagulated by irradiation and its antigenicity reduced over 80% at 10 kGy. Cho et al. (2001) observed by UV spectrum and fragmentation induced by ã-radiolysis. In case of garlic, structural change of irradiation and its antigenicity reduced over 80% at 10 kGy. They reported that the reduction in the antigenicity of BSA following gamma irradiation was caused by the destruction of secondary structure and the exposed the hydrophobic amino acids. Thus, our results indicated that gamma irradiation caused the reduction in allergenicity of PSA because of changes of IgG- and IgE-binding epitopes of PSA by modification of structure. Other food allergens have been also studied for reducing their allergenicity by gamma irradiation.; Lee et al. (2000) reported that major shrimp allergen, tropomyosin, was coagulated by irradiation and its antigenicity reduced over 80% at 10 kGy. Cho et al. (2001) observed by UV spectrum and fragmentation induced by ã-radiolysis. In case of garlic, structural change of irradiation and its antigenicity reduced over 80% at 10 kGy. Therefore, PSA modification by gamma irradiation may be applicable not only for the prevention and treatment of pork allergy, but also for the production of hypoallergenic pork product.

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**References**


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