Food and Chemical Toxicology 51 (2013) 46–52

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Low-dose gamma irradiation of food protein increases its allergenicity in a chronic oral challenge

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ARTICLE INFO

Article history:
Received 26 May 2012
Accepted 11 September 2012
Available online 19 September 2012

Keywords:
Food allergy
Food protein
Food irradiation
Con-A

ABSTRACT

Few chronic food protein models have described the relationship between allergenicity and the molecular structure of food protein after physical processing. The effect of γ-radiation on the structure of food protein was measured by fluorescence, circular dichroism and microcalorimetry. BALB/c mice were intraperitoneally sensitized and then given non-irradiated and irradiated Con-A by daily gavage for 28 days. The tendency to form insoluble amorphous aggregates and partially unfolded species was observed after irradiation. The administration of non-irradiated and irradiated samples at low-dose significantly increased weight loss as well as plasma levels of eotaxin in animals repeatedly exposed to Con-A. Significant lymphocytic infiltrate filling completely the stroma of microvilli and tubular glands was observed in the small intestinal of the group given Con-A irradiated at a low dose. This phenotype was not observed in animals treated with Con-A irradiated at a high dose.

1. Introduction

Food allergy refers to a group of disorders with an IgE-mediated immune response to specific food proteins that affects between 5% of children and 2% of adults (Kagan, 2003). Food allergy has been an important and recurring health problem potentially due to the food consumed becoming increasingly processed (Taylor and Hefle, 2006). Most proteinaceous food allergens are thermostable and resistant to proteases belonging to different groups of plant proteins (Breiteneder and Ebner, 2000). Lectins, also known as plant agglutinins, are a class of proteins that bind to specific moieties of sugar on cells and glycoproteins (Lis and Sharon, 1998). Lectins are found in seeds, especially those of legumes. Some lectins are generally recognized as an important anti-nutrient of food (Shibasaki et al., 1992; Hamid and Masood, 2009). Concanavalin A (Con-A) is a lectin extracted from jack bean (*Canavalia ensiformis*) that has been extensively characterized and widely applied in biotechnology (Edelman et al., 1972; Saleemuddin and Husain, 1991).

Current approaches for the management of allergic disease are based on the removal of specific allergens avoidance diets, drug treatments and preventive measures (Sicherer, 2002). Unfortunately, treatment with medications such as antihistamines and mast cell stabilizers usually play an insignificant role in the treatment of gastrointestinal manifestations (Teitelbaum et al., 2002; Ngo and Furuta, 2005). Genetic engineering offers the opportunity to reduce or even eliminate the compounds in foods that cause allergies (Metcalfe et al., 1996). Nevertheless, associated concerns about so-called cross-contact allergens cannot be eliminated by the incorporation of genetically modified foods. In addition, preventive measures and elimination of allergens are interdependent and has been a target of regulatory agencies around the world (FAO/WHO, 2001).

In this scenario, the development of effective methods to eliminate the antigenic properties of food allergens has been evaluated (Sathe et al., 2005). Among them food irradiation, which is applied to increase the safety and shelf life of foods, has been used (Su et al., 2004). Food irradiation can offer a wide range of benefits to the food industry and the consumer. In recent research, we have

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http://dx.doi.org/10.1016/j.fct.2012.09.011

seen that radiation damages allergens directly by rupturing covalent bonds as a result of transfer of photon energy, or indirectly, by producing reactive oxygen species which can reduce allergenicity by compromising structure–function relationships (Vaz et al., 2012).

Animal models, despite their individual limitations, are useful tools for the study of allergic reactions *in vivo* (Helm, 2002). They have been instrumental in advancing our understanding of the pathophysiological mechanisms for assessing the risk of allergenicity of food products (Knippels and Penninks, 2002). Historically, classic food allergy models have been used to investigate allergenicity of food proteins (Helm, 2002). However, we still need to clarify with the use of other models, if a low dose of radiation attenuates or exacerbates the antigenicity of food allergens when compared to a high dose.

The aim of this study was to establish a chronic allergic mouse model with histological changes in mice and to use the model to examine whether a low dose of ionizing radiation is as effective as a high dose to eliminate the antigenicity of food protein. We monitored lymphocyte migration in the jejunum and representative cytokines of Con-A-induced allergy as well as the structural and molecular profile of irradiated food protein.

2. Material and methods

2.1. Materials

The broad-range molecular weight protein standard, external fluorescence probe 4,4′-bis-1-anilinonaphthalene 8-sulphonate (bis-ANS) and Concanavalin A from *C. ensiformis* (Jack bean) Type IV were purchased from Sigma Chemical Co., USA. All solvents and other chemicals used were of analytical grade from Merck, Germany. All solutions were made with water purified by the Milli-Q system.

2.2. Irradiation of food protein

Con-A in phosphate buffer (pH 7.2) was lyophilized in borosilicate glass vials (16–125 mm) and then irradiated dry under atmospheric O_2 by a Gammacell 220 Excel 60 Co gamma ray irradiator (Ontario, Canada) using doses of 1.0, 10.0 and 25.0 kGy at a rate of 8.25 kGy/h. Each dose was analyzed after irradiation by the following methods.

2.3. Hemagglutinating activity and protein concentration

Hemagglutinating activity (HA), which was defined as the lowest sample dilution that showed hemagglutination, was evaluated as described by Correia and Coelho (1995). Specific HA (SHA) corresponded to the relationship between the HA and protein concentration measured according to Lowry et al. (1951) using bovine serum albumin (BSA) as a protein standard in the range of 0–500 μg/mL. The percentage of the remaining SHA ($\%SHA_{REM}$) was calculated according to the equation: $\%SHA_{REM} = (SHA)_{GM}/(SHA)_{GO} \times 100$, where G_M is the Con-A SHA at each radiation dose (1, 10 and 25 kGy) and G_0 is the SHA of non-irradiated Con-A (control).

2.4. SDS–PAGE

To detect any insoluble aggregates formed, the precipitate was submitted to gel electrophoresis after centrifugation. SDS–PAGE was performed according to Laemmli (1970). Protein samples were resolved on a 10% separating gel and stained with Coomassie blue.

2.5. Chromatography analysis

To detect fragmented protein, the supernatant was separated by RP-HPLC after centrifugation. Irradiated samples were submitted to reverse-phase chromatography on a C-4 column (Vydac-Protein Peptide Ultrasphere) performed on an HPLC system (Shimadzu LC-10AD-Tokyo, Japan) and monitored at 215 nm. The column was equilibrated with 0.1% TFA (solvent A) and eluted using 90% acetonitrile/10% H_2O/0.1% TFA (solvent B) in a non-linear gradient, where B = 0% at t = 5 min; B = 45% at t = 10 min; B = 50% at t = 30 min and B = 100% at t = 35 min.

2.6. Fluorescence and light scattering measurements

Intrinsic fluorescence and light scattering assays were performed on a spectrofluorometer (JASCO FP-6300, Tokyo, Japan). The fluorescence emission intensity of tryptophan from irradiated Con-A was measured in a rectangular quartz cuvette with a 1 cm path-length at 0.1 mg/mL in phosphate buffer, pH 7.2 (25 °C). For intrinsic fluorescence measurements, the excitation was at 295 nm and emission was recorded from 305 to 450 nm, using 5 nm band pass filters for both excitation and emission. For light scattering measurements, the excitation was at 320 nm and emission was recorded from 300 to 340 nm. The light scatted was measured at 90° for the aggregation assays obtained from the area under the fluorescence spectra. The center of spectral mass (CM) was calculated according to Eq. (1): $CM = \sum I_\lambda F_\lambda / \sum F_\lambda$, where F_λ stands for the fluorescence emission at wavelength I_λ and the summation was carried out over the range of appreciable values of F.

2.7. Hydrophobic surface analysis

The hydrophobic surface was measured using the same conditions as employed for the intrinsic fluorescence experiment. Samples were transferred to a quartz cuvette and then mixed with 5 μM bis-ANS. The fluorescence emission spectrum was obtained from 400 to 600 nm, with an excitation at 360 nm (Bhattacharyya et al., 2000).

2.8. Circular dichroism (CD) measurements

CD measurements were carried out on a spectropolarimeter (JASCO J-810, Tokyo, Japan). The instrument was calibrated with D-10-camphorsulfonic acid. The protein concentrations were the following: non-irradiated (5 μM), 1 (5 μM) and 25 kGy (11 μM) in phosphate buffer, pH 7.2. After irradiation, the samples were centrifuged and the measurements were performed with the supernatant. CD spectra were measured in the far-UV range (190–250 nm) in 1 mm path-length quartz cuvette at 25 °C. The data were averaged for 8 scans that were performed at a speed of 50 nm/min and collected in 0.5-nm steps. The baselines (buffer alone) were subtracted from the protein spectra. Results were expressed as mean residue ellipticity, (θ), defined as $(\theta) = \theta_{obs}/(10.C.l.n.)$, where θ_{obs} is the CD in millidegrees, C is the protein concentration (M), l is the path-length of the cuvette (cm) and n is the number of amino acid residues assuming a mean number of 237 residues.

2.9. Differential scanning calorimetry (DSC) analysis

The thermal stability of native and irradiated Con-A was determined by Differential Scanning Calorimetry (DSC). Thermal transitions were monitored by heating using a Microcal VP-DSC micro-calorimeter (Northampton, MA, USA). Prior to all the measurements the buffers and protein solutions were degassed. Thermal transitions were by heating the sample from 5 to 100 °C. A scan rate of 10 °C/h was used. The resulting thermograms were analyzed by the ORIGIN DSC software that was provided by Microcal Inc. (Northampton, USA).

2.10. Sensitization and chronic antigen exposure

Female BALB/c mice (5 weeks old) obtained from Laboratório de Imunopatologia Keizo Asami (LIKA) of the Universidade Federal de Pernambuco were maintained under specific pathogen-free conditions and were given *ad libitum* access to food and water. The animals were kept in an environmentally controlled room, temperature 21 ± 2 °C, under a light/dark cycle of 12 h. Requirements for care and handling of experimental animals according to international and Brazilian regulations (CEEA/UFPE No. 23076.033254/2010-14) were met. All test substances were administered intragastrically by tube. Con-A was dissolved in 0.5 mL of 0.9% sterile saline.

BALB/c mice were immunized subcutaneously on day 0, 15 and 30 using 0.5 ml Con-A (10 μg/mL) dissolved in saline without use of an adjuvant (eight mice per group). Control animals were treated subcutaneously with 0.5 mL saline. Three days before starting the oral challenge in the mice, they were stimulated with the same dose intraperitoneally. During 28 days, mice were treated as follows: group A, immunized mice were treated with 1 mL saline/day; group B, immunized mice were treated with non-irradiated Con-A; group C and D immunized mice were treated with irradiated Con-A at 1 and 25 kGy, respectively. The dose of bean lectin (27 mg/kg body weight/day) was according to total dietary intake of lectins in human subjects consuming vegetarian diets – calculations based on data from Peumans and van Damme (1996).

2.11. Body weight and leukocyte evaluation

Body weight was determined before and after immunization and after oral challenge. The final body weight of each group was obtained from the means of the individual values and expressed in grams (g). Blood samples were obtained and placed into micro-blood tubes containing the anticoagulant ethylenediamine tetra-acetic acid (EDTA). Hematological indices were determined by an automated particle counter, random-access clinical hematologic analyzer Coulter STK-S (Hos-