Food Chemistry 136 (2013) 1141–1147

Contents lists available at SciVerse ScienceDirect

Food Chemistry

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

A potential practical approach to reduce Ara h 6 allergenicity by gamma irradiation

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ARTICLE INFO

Article history:
Received 3 February 2012
Received in revised form 1 September 2012
Accepted 25 September 2012
Available online 2 October 2012

Keywords:
Gamma irradiation
Peanut allergy
Ara h 6
Protein structure
Allergenicity

ABSTRACT

Peanut allergen Ara h 6 was isolated and irradiated at 1, 3, 5, or 10 kGy, and a whole peanut protein extract (WPPE) was also treated by irradiation. Alteration in structure of Ara h 6 was characterised by circular dichroism (CD) spectroscopy, ultraviolet (UV) absorption spectroscopy, fluorescence spectroscopy and SDS–PAGE, and antigenicity was evaluated by immunoblotting and indirect ELISA with anti-Ara h 6 polyclonal antibody. Irradiation induced significant changes in the secondary and tertiary structures of Ara h 6, and the antigenicity of both purified Ara h 6 and WPPE were reduced upon increasing the irradiation doses. Moreover, a good correlation between the loss in α-helix and IgG binding to Ara h 6 was observed. This indicated that irradiation might be an efficient approach to reduce or eliminate peanut allergenicity.

1. Introduction

Peanut allergy is common, frequently severe and typically permanent (Bock, Munoz-Furlong, & Sampson, 2007; Pumphrey & Gowland, 2007; Rona, Keil, Summers, & Gislason, 2007). The prevalence of peanut allergy has been estimated to be between 0.6% and 1% of the US and EU populations (Sicherer, 2002; Tariq et al., 1996). David et al. (1997) have shown that peanut was the third most frequent allergenic food in young Australian and Asian children. Additionally, the incidence of peanut allergy seems to be increasing during recent decades (Ben-Shoshan et al., 2009; Grundy, Matthews, Bateman, Dean, & Arshad, 2002; Sicherer, Munoz-Furlong, & Sampson, 2003). An explanation is that the popularity and consumption of peanut products have increased (Du Toit et al., 2008). Obviously, the investigations on how to prevent it are increasing. Since peanut allergy is mainly triggered by the immunoglobulin E (IgE) recognition of peanut allergens, reducing the allergenic potency or levels of peanut allergens may be an effective approach to lower the allergenic risk. However, previously described methods to reduce the allergenicity of peanut allergens were found to be inefficient, and it was reported that many peanut allergens were resistant to cooking and digesting (Koppelman, Hefle, Taylor, & de Jong, 2010; Mondoulet et al., 2005).

Irradiation has recently become one of the successful techniques to preserve food with minimum interruption of its nutritional and sensory properties (Farkas, 2006; Osterholm & Norgan, 2004). Hundreds of animal feeding investigations of irradiated food have been carried out since 1950, and the studies include subchronic and chronic changes in metabolism, histopathology, function of most systems, reproductive effects, growth, teratogenicity, and mutagenicity. Some of the studies have indicated adverse effects, while no consistent pattern has emerged (Shea, 2000). Consequently, the WHO joint committee concluded that the irradiation of any food commodity up to an overall average dose of 10 kGy presents no toxicological hazard, and toxicological testing of foods so treated is no longer required (WHO, 1988). WHO and FDA have concluded that irradiated food is safe under specific conditions (WHO, 1994, 1999). Moreover, any foods irradiated at levels up to 10 kGy are safe for human consumption without any microbiological hazard and any special nutritional problems (Shea, 2000).

Oh et al. (2009) have evaluated the allergenicity of irradiated peanut extract using splenocytes from peanut-sensitised mice and draw a conclusion that the allergenicity of peanut extracts could be reduced by irradiation treatment, and irradiated peanuts might provide a novel immunogen for an immunotherapy of peanut allergy. It is the first and only report on the gamma irradiation

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http://dx.doi.org/10.1016/j.foodchem.2012.09.091

treatment of peanut allergens, and the structural alteration of irradiation-treated peanut allergens was not defined. However, proteins exposed to irradiation would present structural alterations caused by fragmentation, cross-linking, aggregation, and amino acid modification, affecting their secondary and tertiary structures and their immunoreactivity (Poms & Anklam, 2004). Accordingly, Oh et al. (2009) thought that alteration of epitopes by denaturation of the peanut, after irradiation, might have induced a lower response by T cells. In addition, studies have shown that modification of allergens, by irradiation at levels up to 10 kGy, is a feasible approach to reduce or abolish the allergenicity of egg, milk, wheat and shrimp (Antônio et al., 2012; Lee et al., 2001; Seo et al., 2007; Sinanoglou, Batrinou, Konteles, & Sflomos, 2007). Therefore, before the irradiation process can be generally recommended for reducing allergenicity of peanut allergens, much more information about the process remains to be investigated.

Moreover, Ara h 6 was identified as one of the major peanut allergens, sharing 59% sequence identity and a high degree of structural conservation with Ara h 2, another major peanut allergen (Kleber-Janke, Crameri, Appenzeller, Schlaak, & Becker, 1999; Marsh et al., 2008). Studies in children have demonstrated that Ara h 6 and Ara h 2 were the most commonly recognised peanut allergens, and IgE reactivity to them was a risk factor for the most serious reactions (Flinterman et al., 2007; Pastorello et al., 2001). Currently, there is only one natural counterpart of Ara h 6 and purified Ara h 6 was described by another two independent groups with a molecular weight of approximately 15 and 14.981 kDa, identified by SDS–PAGE and mass spectroscopy, respectively (Koppelman et al., 2005; Suhr, Wicklein, Lepp, & Becker, 2004), and the prepared Ara h 6 needs to be subjected to biochemical and immunological studies. Consequently, our work aims to define the irradiation-induced alterations in structure and antigenicity of a model peanut allergen, Ara h 6. Additionally, studies have shown that the ingredients of the food matrix can have a great impact on the conformation and antigenic properties of proteins during processing (Grimshaw et al., 2003; Lee, Lee, & Song, 2003; Maleki & Hurlburt, 2004). Herein, the antigenic alteration of whole peanut protein extract was investigated to discover whether irradiation processing, under various conditions, could have unpredictable effects on the Ara h 6 allergenicity in the complex food matrices.

2. Materials and methods

2.1. Preparation of whole peanut protein extract (WPPE)

Raw peanuts (Arachis Hypogaea, Xianghua, China) were purchased from a local supplier (Nanchang, China) and were stored at −20 °C until used. After shelling, the skins were removed, and the kernel was ground in liquid nitrogen, using a mortar and pestle. The meal was then defatted by stirring with 5 volumes of precooled acetone for 1 h at 4 °C, and it was recovered by centrifuging at 6000*g* for 20 min at 4 °C. After being defatted three times, the meal was allowed to dry in the air and suspended in 5 vol of 50 mM Tris–HCl (pH 7.2), followed by stirring for 12 h at 4 °C. Then the suspension was centrifuged at 6000*g* for 20 min at 4 °C, and the supernatant was collected as WPPE.

2.2. Preparation of Ara h 6

WPPE (10 mg/ml) was loaded onto a DEAE-Sepharose Fast Flow column (bed dimension, 26 × 300 mm^2, GE Healthcare, USA) previously equilibrated with 50 mM Tris–HCl (pH 7.2, loading buffer) at room temperature. The column was washed with loading buffer until the A_{280} of the effluent was less than 0.2. Proteins were eluted with a linear gradient in a loading buffer (0–0.2 M NaCl in 500 ml of loading buffer) at a flow rate of 1.5 ml/min. Fractions were collected and analysed by SDS–PAGE, and Ara h 6 appeared to be a band of 15 kDa and essentially pure (>95%), calculated by a densitometer scanning of an SDS–PAGE gel stained with Coomassie Brilliant Blue. Further identity confirmation of Ara h 6 was performed with MALDI-TOF-MS fragmentation analysis of selected tryptic peptides with protein-specific sequences (Applied Biosystems, Framingham, MA, USA). Ara h 6 -containing fractions were pooled and concentrated by ultrafiltration with an Amicon Ultra-15 filter with a MWCO of 3.0 kDa (Millipore, USA), and the protein concentration was measured according to the Bradford method.

2.3. Protein content

Protein concentration was determined by the Bradford method, measuring absorbance at 595 nm. Bovine serum albumin was used as a standard.

2.4. Production of polyclonal antibody

Polyclonal antibody against Ara h 6 was produced from New Zealand male rabbits. Before immunising, 1 ml of blood from a non-injected rabbit was collected by the ear-bleeding method as a negative serum blank. Two rabbits were immunised by subcutaneously injecting into their shaved backs 0.2 mg of purified Ara h 6 in Freund's complete adjuvant (Sigma, USA). Subsequent injections of Ara h 6 in incomplete Freund's adjuvant (Sigma, USA) were performed three times, at 2-week intervals, and the immune responses of rabbits were monitored by indirect ELISA. The rabbits were finally bled and each antiserum was separated by centrifuging at 4000*g* for 10 min at 4 °C. All sera were stored at −80 °C until used.

2.5. Gamma irradiation

The purified Ara h 6 (0.2 mg/ml, 30 ml) and WPPE (2 mg/ml, 10 ml) were put into a plastic tube with a cap, and irradiated in a cobalt-60 gamma-irradiator (Jiangxi Academy of Agricultural Sciences, Nanchang, China) in the presence of air. The applied dose levels were 1, 3, 5 and 10 kGy, and the temperature during irradiation treatment was set at 4 °C. Non-irradiated Ara h 6 was used as a control.

2.6. Ultraviolet (UV) absorption spectroscopy

UV absorption spectra analysis was performed by a UV spectrophotometer (UV-VS 2501PC, Shimadzu Corporation, Japan) at room temperature (25 °C). All the samples were homogeneously mixed (vortexed) and scanned from 200 to 400 nm.

2.7. Surface hydrophobicity

Surface hydrophobicity was determined by the 1-anilinonaphthalene-8-sulfonate (ANS) (Sigma, USA). A volume of 20 μl of ANS solution (5 mM in 0.01 M PBS, pH 7.4) was added to 4 ml of Ara h 6 solutions (0.1 mg/ml) and homogeneously mixed by vortex. The relative fluorescence was measured with a spectrophotofluorometer (F-4500, Hitachi, Japan). The excitation wavelength was 390 nm, and the emission wavelength was from 400 to 650 nm at a scanning speed of 1500 nm /min.

2.8. Circular dichroism (CD)

Conformational changes in the secondary structure of irradiated protein samples were analysed using a CD spectropolarimeter (J810, JASCO, Japan). The homogeneously mixed proteins, at a con-