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Effect of high pressure-assisted crosslinking of ovalbumin and egg white by transglutaminase on their potential allergenicity

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ABSTRACT

Transglutaminase (TG) modifies proteins by amine incorporation, crosslinking and deamination. It is used as an important tool in food processing. The aim of this work was to crosslink ovalbumin (OVA) and egg white (EW) with TG, either under high pressure (HP) or under atmospheric pressure on previously HP-treated proteins, to assess the allergenic potential of polymerized allergens. The susceptibility of OVA and EW to TG-mediated crosslinking was enhanced by HP (400 MPa, 40 °C, 30 min). Simultaneous or sequential HP and TG treatments led to the formation of high-molecular weight polymers, but left a substantial amount of monomeric proteins. Results of digestibility experiments, binding to immunoglobulin E (IgE) from egg allergic patients and stimulation of spleen and mesenteric lymph node cell cultures of EW-sensitized BALB/c mice showed that resistance to proteolysis, IgE-binding and immunostimulatory properties of the HP and TG-treated proteins were not substantially different from those of the native ones.

Industrial Relevance: The use of transglutaminase as a crosslinking agent has been suggested for many industrial applications, as in the case of meat, fish, milk or soy proteins, which are good substrates for this enzyme. This work widens the possible use of transglutaminase, for modifying less suitable substrates for crosslinking, as egg proteins, through the use of high pressure before or during crosslinking. In our study, HP-assisted TG-induced OVA and EW crosslinking enhanced gastroduodenal digestion but did not reduce the allergenicity of egg proteins.

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1. Introduction

Food allergy has considerably increased in prevalence over the past decades. Milk, egg, soy and peanut account for the vast majority of food-induced allergic reactions in children, whereas peanuts, tree nuts, fish and shellfish cause most of the food-induced allergic reactions in adults (Burks et al., 2012). Strict avoidance of the responsible food in any of its forms is the only available treatment in many instances. Thus, obtaining new hypoallergenic ingredients derived from allergenic foods to be consumed directly or added to other products would be of great benefit for consumers and stakeholders.

The most commonly used strategy to reduce food allergenicity relies within its own processing (Mills, Sancho, Rigby, Jenkins, & Mackie, 2009). It is well known that processing can either decrease or increase the allergenic potential of food by inducing structural changes that may cause destruction or hiding of the IgE-binding epitopes, but also easier access to epitopes due to protein unfolding. The effect of technological treatments such as heat or hydrolysis has been extensively studied (Jiménez-Saiz, Benedé, Molina, & López-Expósito, 2015); however, other technologies such as enzymatic crosslinking need more research (Heck, Faccio, Richter, & Thöny-Meyer, 2013). Transglutaminase (TG) catalyzes the formation of covalent inter or intramolecular bonds between glutamine and lysine residues, and it is used as an important tool in food processing. TG modifies proteins by means of amine incorporation, crosslinking and deamination, which changes their functional properties, such as gelation aptitude, thermal stability and water-holding capacity (Gauche, Vieira, Ogliari, & Bordignon-Luiz, 2008; Yasir, Sutton, Newberry, Andrews, & Gerrard, 2007), and improves the texture of foods, for instance, seafood, surimi, soy and dairy products (Kieliszek & Misiewicz, 2014).

The effect of TG crosslinking in the immunogenicity and antibody-binding properties of certain allergenic proteins, mainly milk proteins, has been investigated with somehow controversial results. TG crosslinking does not substantially modify the IgE-binding of peanut proteins (Clare, Gharst, Maleki, & Sanders, 2008; Clare, Gharst, & Sanders, 2007), or the IgE- (Stanic et al., 2010) or IgG-binding (O'Sullivan & FitzGerald, 2012) of β-casein. In fact, TG-crosslinked caseinate induces an anaphylactic response similar to that produced by native caseinate in native

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caseinate-sensitized C3H/HeOuJ mice, although it exhibits a reduced sensitization capacity when administered orally to the mice (Van Esch, Gros-van Hest, Westerbeek, & Garssen, 2013). However, Villas-Boas, Vieira, Trevizan, Zollner, and Netto (2010) reported that heated and TG-polymerized β-Lg preserves its sensitizing capacity when administered intraperitoneally to BALB/c mice, whereas skin prick tests showed a significant reduction in the allergenicity of TG-crosslinked β-Lg in cow's milk allergic children as compared with native β-Lg (Olivier, Villas-Boas, Netto, & Zollner, 2012). It should be taken into account that protein stability towards gastrointestinal digestion could also be altered as a result of enzymatic polymerization with consequences for the allergenic potential. Thus, TG-crosslinked β-casein was reported to be more resistant to pepsin digestion than its non-crosslinked counterpart, which is easily hydrolysed by pepsin (Monogioudi et al., 2011; Stanic et al., 2010); although other studies showed a similar rate of hydrolysis of TG-crosslinked sodium caseinate as compared to native caseinate (Havenaar et al., 2013; Jiang & Zhao, 2012).

While certain proteins, such as caseins, are good substrates for enzymatic reaction with TG, other proteins are not, due to their compact structure. In these cases, previous denaturation or addition of reducing agents may increase enzyme access to amino groups (Villas-Boas, Fernandes, Zollner, & Netto, 2012). In that respect, high pressure (HP), an emerging technology that offers excellent opportunities for food product innovation, also provides the conditions that can modify the structural properties of proteins and contribute to their functionality (López-Fandiño, 2006). TG is very stable under HP, showing 50% of residual activity after 12 min at 600 MPa and 40 °C (Lauber, Noack, Klostermeyer, & Henle, 2001a; Menéndez, Rawel, Schwarzenbolz, & Henle, 2006). The combined use of HP and TG treatment allows the crosslinking of proteins, such as β-Lg or lysozyme, which do not react at atmospheric pressure, likely due to protein unfolding that makes glutamine and lysine residues accessible for the TG-catalyzed reaction (Lauber, Krause, Klostermeyer, & Henle, 2003; Partschefeld, Richter, Schwarzenbolz, & Henle, 2007; Schuh, Schwarzenbolz, & Henle, 2010). These observations open new possibilities for modifying the texture and, possibly, the allergenicity of foods.

Ovalbumin (OVA), the most abundant protein in egg white (EW), is considered a major allergen. OVA is highly resistant to proteolysis and it is able to bind IgE from egg-allergic patients following in vitro digestion (Benedé, López-Expósito, López-Fandiño, & Molina, 2014; Martos, Contreras, Molina, & López-Fandiño, 2010). OVA can be modified by TG following heat-treatments for 1 h at 80 °C, but this makes it even more resistant to simulated gastric and duodenal digestion (Giosafatto et al., 2012). The aim of this work was to assess the effects of TG-mediated crosslinking on the digestibility, IgE-binding and immunostimulating properties of OVA and EW as a contribution towards the knowledge of the allergenic potential of polymerized allergens. OVA and EW were treated with TG, either under HP or under atmospheric pressure after being subjected to a HP treatment, to unfold egg proteins and increase their susceptibility to TG-induced crosslinking.

2. Materials and methods

2.1. Transglutaminase-mediated crosslinking of ovalbumin and egg white

OVA grade VI was purchased from Sigma-Aldrich (St. Louis, MO, USA). EW was prepared from fresh eggs, lyophilized and stored at −20 °C and its nitrogen content was determined by the Kjeldahl method (Sosulski & Imafidon, 1990). OVA and EW solutions were prepared at a protein concentration of 5 mg/ml, in Tris–HCl (pH 7.5) and Milli Q water (pH 7.7), respectively. The crosslinking experiments were carried out with a commercial preparation of TG (Activa ®, Ajinomoto Co., Sao Paulo, Brazil), containing 1% of TG and 99% maltodextrin, with 100 U/g of declared activity. The enzyme solution was prepared at a concentration of 2.5 mg/ml in Tris–HCl buffer or Milli Q water, and it was mixed (1:1, v/v) with the OVA or EW solutions, respectively, resulting in a final activity of 50 mU in the reaction mixture. TG-crosslinking experiments were carried out under HP, at 400 MPa and 40 °C for 30 min, and under atmospheric pressure, using native or pre-pressurized (400 MPa, 40 °C for 30 min) OVA and EW as substrates, in a water bath at 40 °C for up to 24 h. As controls, OVA and EW were mixed (1:1, v/v) with Tris–HCl buffer or Milli Q water (1:1, v/v), and either treated at 400 MPa or heated at 40 °C, as explained above.

For HP experiments, samples were vacuum sealed in polyethylene bags avoiding headspace and treated at 400 MPa using a Iso-lab 900 High Pressure Food Processor (Mod FPG7 100:9/2C, Stansted Fluid Power Ltd. Harlow, Essex, UK) with water as pressure-transmitting fluid. The pressure was raised at a rate of 600 MPa/min, maintained for 30 min, and released in less than 4 s. The temperature of the pressure vessel was controlled by a computer program and maintained at 40 ± 2 °C throughout the treatments.

All treatments were performed at least in duplicate and samples were stored at −20 °C until analyses.

2.2. In vitro digestion

Simulated gastrointestinal digestion of the native proteins, HP-treated and crosslinked samples was performed in two different phases. Firstly, a gastric phase with porcine pepsin (EC 3.4.23.1, 3440 U/mg, Sigma), added to the substrate dissolved in water (2.5 mg/ml) at pH 2.0 (adjusted with 2 M HCl), at an enzyme: substrate ratio of 1:20 w/w (172 U/mg of protein) for 1 h at 37 °C. The reaction was stopped by adding 2 M $NaHCO_3$ up to pH 7.0. Secondly, a duodenal phase on the pepsin-digested samples with Corolase PP (AB Enzymes, Darmstadt, Germany), added at an enzyme: substrate ratio of 1:25 (w/w), for 30 min at 37 °C. The reaction was stopped by heating at 95 °C for 10 min.

2.3. SDS-PAGE

Samples of OVA and EW were diluted to 1 mg/ml in a commercial sample buffer (XT Sample Buffer Bio-Rad, Richmond, CA) (non-reducing conditions) or in the same buffer containing 5% (v/v) β-mercaptoethanol (reducing conditions) and heated at 95 °C for 10 min. Samples were analyzed on Precast Criterion XT 12% Bis–Tris gels (40 μg of sample per well) and electrophoretic separations were carried out at 100 V, using XT-MES as running buffer. Gels were stained with Bio-Safe Coomassie G-250 (all from Bio-Rad).

2.4. RP-HPLC

RP-HPLC analysis was performed using a Waters 600 HPLC instrument (Waters, Milford, MA) with a RP318 column (250 × 4.6 mm, Bio-Rad). Operating conditions were as described by Jiménez-Saiz, Belloque, Molina, and López-Fandiño (2011).

2.5. Human IgE binding by inhibition ELISA

Human IgE-binding of native, HP-treated and crosslinked OVA and EW and their digests was assessed by inhibition ELISA as previously reported (Jiménez-Saiz, López-Expósito, Molina, & López-Fandiño, 2013), using a pool of 7 different sera from egg allergic children with IgE specific to OVA and EW ranging between 35–1326 kU/L and 66–2140 kU/L, respectively.

2.6. Cytokine secretion and cell viability of mice spleenocytes and mesenteric lymph node cells

Female BALB/c mice (n = 10) were sensitized to EW by oral gavage (5 mg of protein plus 10 μg of cholera toxin – List Biologicals, Campbell, CA – per mouse), as described by Jiménez-Saiz, Rupa, and Mine (2011). After sacrifice, whole spleens and mesenteric lymph nodes (MLN) were separately pooled in two groups (n = 5) and cell suspensions were

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