Methods 66 (2014) 34–43

Contents lists available at ScienceDirect

Methods

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

Production and analysis of recombinant tree nut allergens

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ARTICLE INFO

Article history:
Available online 31 July 2013

Keywords:
Tree nut
Allergy
Recombinant allergen
Epitope
Food allergy

ABSTRACT

Allergic reactions to tree nuts are a growing global concern as the number of affected individuals continues to rise. Unlike some food allergies, tree nuts can cause severe reactions that persist throughout life. The tree nuts discussed in this review include those most commonly responsible for allergic reactions: cashew, almond, hazelnut, walnut, pecan, Brazil nut, pistachio, and chestnut. The native allergenic proteins derived from tree nuts are frequently difficult to isolate and purify and may not be adequately represented in aqueous nut protein extracts. Consequently, defined recombinant allergens have become useful reagents in a variety of immunoassays aimed at the diagnosis of tree nut allergy, assessing cross-reactivity between various nuts and other seeds, mapping of IgE binding epitopes, and analyzing the effects of the food matrix, food processing, and gastric digestion on allergenicity. This review describes the approaches that can be used for the production of recombinant tree nut allergens and addresses key issues associated with their production and downstream applications.

1. Introduction

Food allergies are adverse immunological (hypersensitivity) reactions to normally harmless proteins in foods [1]. The specific proteins which are recognized by IgE and elicit an immune response are known as allergens. The average human diet contains a wide variety of foods; however, only a few are responsible for the majority of food allergies [1]. The most common allergenic foods include milk, egg, wheat, soy, fish, peanuts, tree nuts, and shellfish. Often allergies to wheat, egg, milk, and soy are outgrown whereas allergies to peanuts, shellfish, and tree nuts typically persist throughout life and can cause severe type I (IgE-mediated) allergic reactions [2–5]. Tree nuts are typically eaten as snacks or incorporated into foods, and include almond, Brazil nut, cashew, chestnut, coconut, hazelnut, macadamia, pecan, pine nut, pistachio, and walnuts. The popularity and consumption of tree nuts varies among populations and geographical locations. The most commonly consumed tree nuts in the US, in rank order, are cashew, almond, pistachio, pecan, and walnut, whereas in Europe the most popular nuts, in rank order, are walnuts, almonds and hazelnuts [6–8]. Allergic reactions to tree nuts are rising; it is currently estimated that 0.6% of the US population and up to 1.4% of the European population are allergic to one or more tree nuts [9–11].

Food allergens are classified as either type 1 or type 2 according to their specific biochemical and immunological properties. Type 2 allergens are referred to as incomplete allergens. Reactivity to these food allergens often results from a primary sensitization by aeroallergens (e.g., pollens) [12–14]. The subsequent consumption of foods, including a variety of fruits and vegetables containing proteins homologous to the sensitizing allergen, triggers an IgE-mediated reaction [14,15]. Such reactions are frequently mild and localized to the oral cavity.

Type 1 allergens, in contrast, are known as complete food allergens as they both sensitize the patient, primarily through the gastrointestinal tract (GIT), and elicit allergic symptoms. Type I allergens are typically resistant to thermal denaturation and gastric digestion, consequently retaining a substantial proportion of their IgE-reactive epitopes [12,15,16]. Common foods containing type 1 food allergens include peanuts, tree nuts, egg, milk, and fish [15,17]. To date several type 1 allergens have been identified in tree nuts including hazelnut Cor a 8, 9, 11, 12, and 13, and walnut Jug r 1, 2 and 3. It should be noted that while type 1 allergens tend to predominate in peanut and tree nuts, these foods have been found to contain type 2 food allergens as well (e.g., chestnut Cas s 1, hazelnut Cor a 1 and 2, and almond Pru du 4), a likely result of patient sensitization to cross-reactive pollen allergens [15,17]. The higher incidence of type 1 food allergy in children is likely due to a greater degree of intestinal permeability and a less developed immune system [15]. Symptoms of type I food-induced allergy range from mild gastrointestinal reactions, such as abdominal pain, diarrhea, and vomiting, to severe life threatening respiratory reactions, such as systemic anaphylaxis [15]. It is estimated

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http://dx.doi.org/10.1016/j.ymeth.2013.07.033

that 8–50 per 100,000 individuals experience an anaphylactic reaction annually [18–24]. In children, peanut and cashew nut are the most common eliciting agents [21,22]. Due to the severity of reactions to tree nuts and the growing number of individuals affected, extensive research has been performed to identify and characterize constituent IgE-reactive proteins. In addition, immunoassays aimed at investigating cross-reactivity between various nut and seed proteins, as well as structural studies, IgE epitope mapping, and analyses of the effects of associated food matrices, enzymatic digestion, and denaturation have been preformed in hopes of determining common features which may predispose such proteins to be targeted by IgE. It is anticipated that the accumulating information will provide insights into the unique features which render tree nuts allergenic and may be useful for diagnostic and therapeutic purposes.

2. Tree nut allergens

To date, 32 tree nut proteins have been shown to be reactive with IgE from allergic patients (http://www.allergen.org/). Many of these proteins have been extensively studied at the molecular level. A current list of officially designated tree nut allergens is presented in Table 1. These allergens can be grouped based on their sequence, function, and structural similarities [25,26].

2.1. Seed storage proteins

One such group is the seed storage proteins, which are estimated to account for ~50% of the total protein in most nuts [27–31]. Seed storage proteins act as a carbon and nitrogen source during germination and serve as amino acid reserves for the developing seedling [32,33]. Those with allergenic properties include the 11S globulins (legumin-like proteins), 7S globulins (vicilin-like proteins), and 2S albumins [25,34]. The 11S globulins are members of the cupin superfamily characterized by a six stranded β–barrel conformation [35,36]. They are typically ~360 kDa hexamers with each subunit containing an acidic 42–40 kDa polypeptide that is disulfide-linked to a ~20 kDa basic polypeptide [26,33,37]. It is not uncommon for multiple 11S globulin isoforms to be expressed in nuts. Surprisingly, despite sequence similarity, not all isoforms in a given nut display similar IgE reactivity [33,38,39]. The 7S globulins are also members of the cupin superfamily. They are typically trimeric with a molecular mass of ~150 kDa comprised of 40–55 kDa polypeptides [25,33,40]. The 2S albumins are small ~13 kDa protein members of the prolamin superfamily. They contain eight conserved cysteine residues which form two intrachain disulfide bonds and two interchain disulfide bonds linking a ~9 kDa and ~4 kDa polypeptide chain [26,33,41–43].

2.2. Pathogenesis-related proteins

Several pathogenesis-related proteins (PR-proteins) have been identified as tree nut allergens. PR-proteins are produced in the plant upon attack by bacteria, fungi, or when abiotically stressed [44,45]. They range from 5–70 kDa, are stable at acidic pH, and tend to be highly resistant to proteolytic degradation [44,45]. Allergenic PR-proteins include chitinases, Bet v 1 homologues, and lipid transfer proteins (LTP) [46–50].

2.3. Structural proteins

Another group of tree nut allergens are the profilins. These are small cytosolic ~12–15 kDa proteins that bind actin in eukaryotic cells and perform an important role in regulating actin filament polymerization [51,52]. Profilins are highly conserved among plants, displaying both structural and sequence similarities (70–85% amino acid sequence identity) [25]. These proteins have been described as pan-allergens because of the considerable cross-reactivity observed between profilins from different sources [25,35,53,54]. Oleosins are small ~15–26 kDa hydrophobic proteins that serve to stabilize oil bodies in desiccated seeds [55–57]. They have a highly conserved structure with three distinctive domains; a hydrophilic domain at the N-terminus, a central hydrophobic α-helical domain, and an amphipathic α-helical domain at the C-terminus [55,58]. Among the tree nuts, oleosin has only been identified as an allergen in hazelnuts [59].

2.4. Other allergenic proteins

In recent years several additional proteins have emerged as potentially important allergens in tree nuts including manganese superoxide dismutase (MnSOD), 60S acidic ribosomal protein P2, and cytosolic class I small heat shock protein. MnSOD is a homodimeric or a homotetrameric enzyme that is located in mitochondria and peroxisomes [60,61]. In plants, MnSOD plays an important role in preventing cellular damage by reactive oxygen species [60]. MnSOD was identified as a minor allergen in pistachio nuts though it has been suggested that the high degree of sequence similarity between MnSOD from different organisms could potentially lead to allergen cross-reactivity [62]. The 60S acidic ribosomal protein P2 is a small ~11 kDa ribosomal protein with a highly conserved structure that is involved in mRNA translation [63–66]. Originally identified as minor allergens in several fungi [67–70] they were recently recognized as an IgE-reactive protein in almond nut [71]. Finally, the cytosolic class I small heat shock proteins are produced following abiotic stress [72,73] and are reactive with IgE in some chestnut-allergic sera (unpublished data, http://www.allergen.org/).

3. Purification of native tree nut allergens

The growing number of allergens identified in tree nuts is mainly due to advances in protein purification and molecular biology techniques. Initially, studies to identify tree nut allergy and constituent IgE-reactive proteins were performed using aqueous nut extracts in immunoblotting assays, radioallergosorbent tests, or skin prick tests [74–83]. Further characterization has been accomplished by application of a wide variety of biophysical techniques such as high performance liquid chromatography, mass spectrometry, nuclear magnetic resonance, X-ray crystallography, amino acid sequencing, circular dichroism spectroscopy, and enzymatic activity assays [37,84–93]. Application of these techniques has led to the identification, characterization and, in some cases, purification of specific allergens, which can subsequently be used in immunoassays for the detection of IgE from tree nut allergic individuals.

Whereas native allergens offer the conformationally and antigenically optimum choice for analysis, the characteristics of some tree nut allergens complicate their use in analytical studies [85,94]. First, native allergens are often difficult to purify to homogeneity even when applying multistep protocols [28,30,95–98]. Enzymatic degradation during *in vivo* (*in planta*) synthesis, fractionation or purification, the frequent presence of multiple isoforms, strain/cultivar differences, variations reflecting different developmental stages, and environmental influences on growth, can all impact the degree of homogeneity within and between samples [85,94]. A lack of homogeneity can interfere with the downstream use in diagnostics and immunoassays. Finally, some native allergens are present at low concentrations in nuts necessitating the use of excessive starting material and complex purification