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Stability of allergens

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ABSTRACT

For proteins to cause IgE-mediated allergic reactions, several common characteristics have to be defined, including small molecular size, solubility and stability to changing pH levels and enzymatic degradation. Nevertheless, these features are not unique for potent allergens, but are also observed in non-allergenic proteins. Due to the increasing awareness by regulatory authorities regarding the allergy pandemic, definition of characteristics unique to potent allergens would facilitate allergenicity assessment in the future. Despite major research efforts even to date the features unique for major allergens have not been elucidated so far. The route of allergen entry into the organism determines to a great extent these required characteristics. Especially orally ingested allergens are exposed to the harsh milieu of the gastrointestinal tract but might additionally be influenced by food processing. Depending on molecular properties such as disulphide bonds contributing to protein fold and formation of conformational IgE epitopes, posttranslational protein modification or protein food matrix interactions, enzymatic and thermal stability might differ between allergens. Moreover, also ligand binding influences structural stability. In the current review article, we aim at highlighting specific characteristics and molecular pattern contributing to a stabilized protein structure and overall allergenicity.

1. Introduction

Since many years analyzing common allergen characteristics has been a major research focus worldwide. To answer the question what makes certain proteins to become allergens, the terms *allergy* and *allergens* have to be well defined. As proposed by an expert panel of the World Allergy Organization (WAO) in 2003, an allergic response is defined as a hypersensitivity reaction with objectively documented symptoms triggered by immunological mechanisms, which is initiated by an IgE- or non-IgE-mediated response (Johansson et al., 2004). Due to essential mechanistic differences only the impact of protein stability in the context of IgE-mediated response will be discussed in this review. Referring to IgE-mediated allergic reactions, the term *allergen* describes an antigen triggering an allergic response by IgE binding after initial sensitization (Aalberse, 2000). Based on this definition the most important property of allergens distinguishing them from non-allergenic proteins is the sensitizing capacity in susceptible subjects (Aalberse, 2000). Moreover, also the elicitation of an allergic immune response is an important characteristic of allergens (Masilamani et al., 2012). To trigger an allergic immune response, allergens have to come in contact with immune cells via mucosal body surfaces or the skin. In scientific literature, allergen characteristics such as solubility, stability, as well as molecular properties and molecular size have been repeatedly reviewed and summarized (Aalberse, 2000; Huby et al., 2000). Thus, specific protein properties are essential to reach the organ specific immune induction sites.

2. Implications of allergen stability based on organ specific entry routes

Allergens get access to the human immune system by two different entry pathways, i.e. via the skin or via mucosal surfaces (Dunkin et al., 2011). Depending on the location of allergen uptake, specific molecular properties, which in part are linked with allergen stability, are required to enable transport through epithelial barriers and interaction with organ resident immune cells. In turn, requirements for allergens stability can be allocated to different organ systems based on knowledge regarding specific characteristics of different entry routes (Fig. 1). *Allergen stability* can be defined as the ability of proteins to preserve their native, three-dimensional pattern after chemical, physical or protease treatment over time (Breiteneder and Mills, 2005; Deller et al., 2016).

Allergens entering the organism via the skin have to be able to cross the epidermal barrier. Between the outer layer of corneocytes, lines of intercellular lipids form hydrophobic and hydrophilic structures

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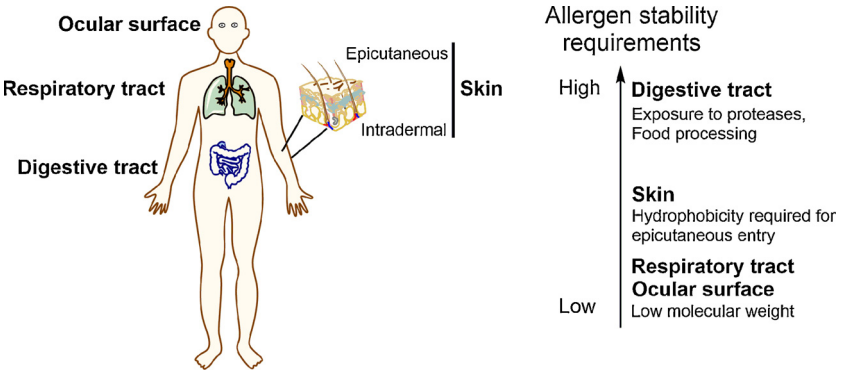

Fig. 1. Allergen uptake via skin and mucosal surfaces. To survive the compartment specific environment, different stability requirements enable allergens to penetrate epithelial barriers and to interact with immune cells for induction of an allergic response.

modulating skin permeability (De Benedetto et al., 2012). Thus, allergens penetrating outer barrier of intact skin have to show hydrophobic properties or need binding to a lipophilic carrier. Due to lack of protein degrading enzymes no specific enzyme stability is required in this compartment. Furthermore, the need for specific stability properties of intradermally injected allergens such as insect venoms is even lower as after injection no further barrier has to be crossed (Fig. 1).

Inhalant allergens, such as pollen, house dust mite proteins or spores, do not require specific stability to low pH levels or to enzymes. Upon inhalation allergens need to exhibit extensive solubility in the aqueous milieu of the mucosal surfaces and the correct particular size to enter and escape mucus binding properties, as both mucus and tissue of the respiratory tract might act as an allergen reservoir (Rimmer et al., 2015). Tear film mucins show similar functional properties, due to their genuine role to prevent antigen and pathogen interaction with the ocular surface (Hodges and Dartt, 2013). Of interest, the proteolytic activity of allergens themselves seems to confer enhanced sensitizing potential by disruption of epithelial barrier layers as it has been shown e.g. for house dust mite allergens (Wan et al., 1999).

Allergen sensitizing via the gastrointestinal tract require high protein stability to ensure proteolytic and hydrolytic resistance (Breiteneder and Mills, 2005). If food processing precedes ingestion, major allergens triggering an allergic response via the oral route even have to show heat resistance. Due to the high stability requirements for proteins triggering an allergic response after oral ingestion, this article will primarily focus on this route of allergen exposure to highlight the relevance of protein stability in allergic disease.

3. Characteristics of food allergens associated with protein stability

When it comes to allergens triggering an allergic response via the oral route, several specific characteristics have been described in the scientific literature. Only few proteins account for most food allergies and many food allergens share some common characteristics, which nevertheless are not unique to them (Bannon, 2004; Breiteneder and Mills, 2005; Vickery et al., 2011). Food allergens are usually relatively abundant in the food source, which seems to enhance their chance to interact with the immune system (Bannon, 2004). Allergenic food proteins are generally relatively small with a molecular size below 70 kDa (Vickery et al., 2011) However, a molecular size of at least 3.5 kDa is necessary to induce an antibody response, as smaller fragments of e.g. digested β-lactoglobulin (BLG) were no longer immunoreactive (Bogh et al., 2013). For a long time food allergens were typically classified into two groups. Class I allergens or complete allergens were described to sensitise via the oral route (Bannon, 2004). This group included allergens found in peanut, milk or wheat, which were accepted to elicit severe, generalized symptoms. Class II allergens, also known as non-sensitising elicitors or incomplete allergens, were revealed to share homologies with aeroallergens (Aalberse, 1997; Vieths et al., 2002). Sensitisation was defined to occur indirectly towards cross-reactive aeroallergens via the respiratory route. Class II allergens were considered to be associated with milder, local symptoms such as the oral allergy syndrome (Webber and England, 2010).

In line with this classification, frequently found linear IgE binding epitopes seem to play an important role in food allergy as conformational epitopes are lost during the passage through the gastrointestinal tract (Bannon, 2004). Especially in milk and egg allergy, the presence of IgE recognizing linear epitopes was described to be associated with reactions to heated allergens and persistence of allergic symptoms (Chatchatee et al., 2001; Jarvinen et al., 2007). However, the ratio of recognised linear versus conformational epitopes was shown to vary between different milk allergens α-lactalbumin (ALA), β-casein and BLG (Madsen et al., 2014). Thus, the sensitising capacity after gastrointestinal digestion may depend on the one hand on allergen stability to proteolysis during passage through the digestive tract and on the other hand on the abundance of linear epitopes with immunostimulating capacity, as conformational epitopes may get destroyed.

4. Gastrointestinal physiology or food associated factors influencing allergen stability

4.1. Stability to gastrointestinal protein digestion

As outlined above, one of the main characteristic of proteins triggering an allergic response via the gastrointestinal tract seems to be resistance to gastrointestinal digestion. Protein digestion is influenced by several factors and consequently varies among different individuals. Digestion of ingested proteins starts with mastication and maceration in the oral cavity. Breaking food into smaller particles facilitates the subsequent gastrointestinal enzymatic digestion. In the oral cavity the enzymes α-amylase, secreted by the salivary glands, and lingual lipase start enzymatic degradation. The cleavage of 1,4-glycosidic bonds of carbohydrates by amylase affects allergens, as most are glycoproteins (Fig. 2) (Boehlke et al., 2015). After this initial oral digestion, ingested proteins are swallowed. Following a quick esophageal passage, proteins enter the stomach and denature due to the acidic gastric fluid. Besides denaturation, a low gastric pH is essential for pepsin activation. At low pH levels, pepsinogen, the inactive zymogen secreted by the parietal cells, gets activated. Moreover, unfolded proteins are more susceptible to enzymatic hydrolysis due to exposed cleavage sites. With an enzymatic optimum between pH 1.8–3.2, pepsin, an aspartic protease, hydrolyses proteins preferentially, but not exclusively, between hydrophobic and aromatic amino acid residues phenylalanine, tyrosine, tryptophan and leucine (Oka and Morihara, 1970; Tang, 1963; Trout and Fruton, 1969). In fasted state, gastric fluids of healthy adults are acidic with a pH between 1.5 and 3 and with a pepsin concentration between 0.11–0.22 mg/ml increasing up to 0.58 mg/mL after eating