



Proteomics and its impact on food allergy diagnosis



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ABSTRACT

Food allergies are a relevant health problem and symptoms range from mild to severe life-threatening reactions. With the help of up to date proteomics the causative food allergens can be identified from individual food sources. A short overview on the application of proteomics to assess the physicochemical properties of food allergens is presented.

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1. Food allergies – a major health concern

Allergic diseases are regarded as a relevant health problem in our society. Allergic or hypersensitivity reactions to foods are triggered by the immune system, while food intolerances are due to lack of enzymes or induced by pharmacologically active substances in the food. Among the immune mediated hypersensitivity reactions, type 1 allergy is mediated by specific IgE antibodies directed against food components. In type 4 allergy symptoms are caused by cell mediated reactions (e.g. celiac disease, food protein-induced enterocolitis) [1]. It is assumed that around 6–8% of children and up to 3% of adults are affected by allergic reactions to food. In the following only type 1 (IgE mediated) food allergies will be discussed. Symptoms caused by food intake range from mild local up to generalized reactions, which in some patients are life-threatening. The most important food sources causing allergic reactions are milk, egg, peanut, tree nuts, sesame seed, fish, seafood, fruits and vegetables. Milk and egg are predominant allergen sources in childhood, while other foods such as peanut, tree nuts and sesame seeds are relevant for both, children and adults.

2. Food allergens

In the last two decades great efforts were undertaken to identify the allergenic proteins from plant derived and animal foods, to study their physicochemical characteristics and their interaction with immune cells. Consecutively, allergen databases were built and maintained to provide extensive information about allergens (e.g. www.allergen.org). It became evident, that only a minority of all known protein families contain food allergens [2,3]. With regard to plant food allergens the most relevant protein families are the 2S albumins, seed storage proteins identified from seeds (e.g. sesame seeds, sunflower seeds . . .) and nuts (e.g. hazelnut, walnut, brazil nut . . .) and peanut, followed by the non-specific lipid transfer protein (nsLTPs) representing the major allergens in fruits like peach and apple and tree nuts [3]. Furthermore, the seed storage proteins from the cupin superfamily, 7/8S globulins and 11S globulins from seeds, tree nuts and peanuts are well-defined food allergens. The ubiquitous protein family profilin contains allergens that are allergenic in a range of plant-foods. Another protein family, the Bet v 1 related proteins are present in both, monocot and dicot plants, and seem to exert a range of important functions within the plant, including upregulating resistance when pathogen attacks or environmental stress affects the plant.

Among the animal food sources tropomyosin and EF-hand proteins are the most prevalent protein families. Tropomyosins from crustaceae, molluscs and fish parasites are causing allergic symptoms. An EF-hand domain is shared by a number of different Ca²⁺-binding proteins, and enables either Ca²⁺ dependent signaling and/or Ca²⁺ buffering or transport. The major allergen from fish, parvalbumin, is present in the majority of fish species. The caseins,

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mammalian proteins in milk account for a high percentage of allergic symptoms in milk allergic patients.

3. How to assess the quality of purified food allergens?

In order to use well defined and highly pure allergen batches for diagnostic application several parameters need to be assessed applying up to date technology. Allergens can be purified either from natural sources or as a recombinant protein from heterologous expression systems. For each protein suitable purification protocols have to be established and the final batch needs to be assessed with regard to purity, correct primary sequence and structural features equivalent to the native protein. Furthermore, the enzymatic activity (if known) should be investigated in the purified protein as well as the allergenic activity. Within the EU project Europrevall an allergen library was established as a proof of concept [4,5]. This library comprised a total of 53 food allergens derived from either natural or recombinant sources. In parallel, a list of quality parameter was agreed and the methods to assess the physicochemical characteristics listed. It became evident that a range of methods should be applied to assess purity, folding and allergenic activity. For example, purity can be assessed by SDS-PAGE. However, additional information can be obtained when applying 2D electrophoresis, HPLC or capillary electrophoresis detecting individual isoforms, differing in only a few amino acid residues. For verifying the molecular masses of proteins mass spectrometry has proven to be a powerful tool with MALDI and ESI being the most commonly used ones [6]. For example allergens from rice were separated by 2D electrophoresis and subsequently analysed by MALDI-TOF MS [7]. With different approaches posttranslational modifications can be spotted but also differences in isoforms and by MALDI-in source decay sequencing of peptides and subunits can be performed as it has been shown for hazelnut allergen, Cor a 14 [8]. Recently allergic reactions directed against alpha galactose-1,3-galactose have gained interest as a new sensitizing source, present on mammalian meat. For the detailed characterization of these residues LS-MS/MS applied after 2D electrophoresis facilitated the characterization of this immunogenic posttranslational modification of beef proteins [9].

To study the secondary structural elements of a given protein circular dichroism is a fast and non-destructive technique. However, for more detailed studies to assess the structural and dynamic properties of proteins at atomic level, NMR analysis provides a deeper insight. In a first approach 1D¹H NMR analysis was applied for the allergens in the Europrevall library and provided additional information with regard to structural changes during heat treatment [10]. For more refined analysis 2D NMR is recommended as well as X-ray crystallography [11]. With regard to allergenic activity immunoassays are performed investigating the IgE-binding activity of the purified protein batch by ELISA or western blotting techniques, usually applying allergic patients' sera. In addition cellular assays can be performed, such as the basophil histamine release assay or T cell proliferation assays.

4. Well characterized food allergens for diagnosis

So far, no approved immunotherapies are available for food allergies. Avoidance of the causative food is the method of choice. Therefore, reliable diagnosis contributes to better advice for the patient with regard to diet.

Up to now, total food extracts lacking standardization are in use for routine diagnosis to perform both, skin prick tests and in vitro serum IgE testing [12]. However, during the last decade single allergen testing became available. Testing based on single well defined allergen batches gives additional diagnostic value with regard to assessing the patients' allergic sensitization to a limited

number of allergens and helps to determine the range of cross reactivity to other potential allergen sources. This in turn reduces the risk of unnecessary exclusion diets and contributes to the patient's quality of life.

5. Prevention of food allergy

As mentioned above there is no causative treatment of food allergy and avoidance of the allergen source is recommended. However, allergen labelling on food products sometimes lacks the required information with regard to allergen content. Therefore, food industry and regulators undertook efforts to develop strategies for best practice for allergen risk assessment, to examine training strategies for food manufacturing companies and to identify the relevant analytical tools [13]. It is expected that the application of various mass spectrometry approaches may provide useful information to assess threshold levels of individual allergens present in both, native and processed foods. Recently, Koeberl et al. developed a multiple reaction monitoring mass spectrometry (MRM-MS) to quantify allergens and/or marker proteins specific for each food [14]. Another example of quantifying 10 allergens present in different soybean varieties down to 0.5–0.7 µg/mg was shown by Houston et al. [15]. Absolute quantification of proteins in foods contributes to assess the risk of allergen levels the allergic consumer will be exposed to and allow the development of more refined and standardized detection assays to improve allergenic risk management in the food industry.

6. Conclusions and outlook

Application of up to date proteomics facilitates to characterise food allergens to be used for diagnostic application and is superior to extract based approaches. This in turn contributes to patient tailored diagnosis, improved patient management such as dietary recommendations, avoidance strategies, and helps food industry to fine tune their in house quality standards and determine threshold limits for the allergic consumer. Moreover, risk assessment strategies need to be developed which can be used for well-established food production as well as for novel food processing regimens. Also novel food sources including new varieties and food sources so far not used should be tested whether they pose a potentially increased allergenic risk. These questions are now addressed in an EC funded COST Action "IMPARAS".

Within this Cost Action, a multidisciplinary approach was developed including proteomic specialists, immunologists, nutritionists and allergists to tackle burning questions of allergenic risk assessment for known food sources, for novel foods and novel food processing strategies and their impact on allergenicity of individual food proteins.

Conflict of interest

None.

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