Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. 78 (3) 315–320 (2013)
JSCS–4418
UDC 616–056.3:641.3:66.0:577.1+54.061+615.37
Survey

SURVEY

Authentication of food allergens

KARIN HOFFMANN-SOMMERGRUBER*

Department of Pathophysiology and Allergy Research, University of Vienna, Austria

(Received 22 October 2012, revised 2 January 2013)

Abstract: Pure allergen batches are required for precise personalised diagnosis of food allergies. Furthermore, they can be used to develop sensitive allergen detection assays in foods and are valuable tools to develop novel immune-therapies. However, these reagents have to be well characterised and have to meet certain quality criteria. Within the EU-Project EuroPrevall, the concept of an allergen library comprising the most important food allergens from animal- and plant-derived foods was developed together with a catalogue of physico-chemical and immunological properties that had to be investigated. In close cooperation, partners from academia and the biotech industry applied well-established laboratory techniques as well as novel high throughput assays to analyse the most important features of the final protein batches. It is expected that this proof of concept will contribute to improved authentication of allergens for both routine application in allergy diagnosis and treatment and risk assessment in food production.

Keywords: food allergens; IgE; diagnosis.

CONTENTS

1. INTRODUCTION

Allergies are regarded as the epidemic of the present century affecting a steadily increasing number of people. It is usually harmless, non-toxic proteins that induce an immune response in predisposed individuals, evoking symptoms that range from mild and unpleasant local reactions to generalized and even life threatening conditions.

Up to now, no immunotherapy for food allergies is available; therefore avoidance of the incriminating food source is the method of choice.

* Corresponding author. E-mail: Karin.hoffmann@meduniwien.ac.at
doi: 10.2298/JSC121022002H

Great efforts have been undertaken to identify and characterise non-toxic food proteins that induce an IgE-mediated allergic response in patients. Many hundreds of allergens from animal- or plant-derived food have been identified in the last 2 decades. The information on these proteins, regarding their physicochemical properties and their allergenic relevance, including cross reactivities, has been entered into allergen databases. Of these, the IUIS allergen database (http://www.allergen.org/) provides the systematic nomenclature for allergens and their respective allergen designations. Entries for new allergens are only accepted upon approval of the allergen nomenclature subcommittee. Based on these datasets, it became clear that proteins with allergenic activity are restricted to a limited number of protein families and are not randomly distributed.[1] Despite this, it remains to be clarified what intrinsic factors contribute to the allergenic activity of certain proteins.

2. ALLERGEN PRODUCTION AND AUTHENTICATION

Purified food allergens are applied for *in vitro* diagnosis (component resolved diagnosis, CRD) and as reference materials for the quantification of the respective allergen in foods.[2] Furthermore, they are relevant tools to study the function–structure relationship of food allergens and the impact of food processing and the food matrix on the allergenicity of individual allergens. Finally, these well-characterised molecules are used to develop novel vaccination strategies for food allergies.

For each of these applications, a quality catalogue for purified food allergens has to be developed to harmonise the purification strategies and to enable a correct analysis of the obtained data.

Within the EU-funded project EuroPrevall, partners from academia and the biotech industry worked together to establish a food allergen library comprising the most important allergens from animal and plant derived food sources (Table I).[3] Purified allergens from cow's and goat's milk, hen's egg, fish, shrimp, fruits from the Rosaceae family (apple, peach), hazelnut, peanut and celery were included.[4] The majority of these are already in use for component resolved diagnosis and others are not yet included. These well-defined allergens were used to compare conventional *in vitro* diagnosis with CRD, to set up *in vitro* models of food processing and to develop novel allergen detection assays in foods.

The individual allergens were purified either from natural sources or produced as recombinant proteins. Prior to inclusion into the allergen library, a purified allergen had to meet several quality criteria. As the most important quality criteria, purity and identity, protein folding, post-translational modifications and immunochemical properties were identified.[5] The most important quality parameters and the analytical applied methods are summarised in Table II.

Individual allergens can occur as a single isoform or as a range of highly similar proteins with varying IgE binding activity. In addition, the abundance of a given allergen may influence the decision of whether to produce the protein in a heterologous expression system or to purify the single protein or a mixture of isoforms from natural sources. For both approaches, purity and verification of the correct sequence are prerequisites for further applications. As a routine method, 1D-gel-electrophoresis (SDS-PAGE) was applied and only protein batches with purity > 95 % were further analysed. Usually a combination of methods was used to assess purity, *i.e.*, HPLC and capillary electrophoresis. For natural allergens comprising a range of isoforms, 2D-gel electrophoresis was performed to identify the number of isoforms. To verify the correct *N*-terminal amino acid sequence, Edman degradation and mass spectrometry were performed to verify the correct mass data.

TABLE I. EuroPrevall Allergen library. The most important food allergens from animal and plant food sources were identified and either purified from natural sources or produced as recombinant proteins

Source		Protein family/functional properties	IUIS Allergen designation[a]	Origin
		Animal food allergens		
Cow's milk	*Bos domestica*	α-Lactalbumin	Bos d 4[b]	Cow's milk (raw)
		β-Lactoglobulin	Bos d 5[b]	Cow's milk (raw)
		Total casein	Bos d 8[b]	Cow's milk (raw)
Goat's milk	*Caprinus domesticus*	Total casein		Goat's milk (raw)
Hen's egg	*Gallus domesticus*	Ovomucoid	Gal d 1[b]	Hen's egg white
		Ovalbumin	Gal d 2[b]	Hen's egg white
		Ovotransferrin	Gal d 3[b]	Hen's egg white
		Lysozyme	Gal d 4	Hen's egg white
		Serum albumin	Gal d 5[b]	Hen's egg yolk
Carp	*Cyprinus carpio*	Parvalbumin	Cyp c 1[b]	Recombinant (*Escherichia coli*)
Codfish	*Gadus morhua*	Parvalbumin	Gad m 1[b]	Codfish muscle and recombinant (*E. coli*)
Shrimp	*Penaeus aztecus*	Tropomyosin	Pen a 1[b]	Recombinant (*E. coli*)
		Plant food allergens		
Apple	*Malus domestica*	Bet v 1 homologue, PR-10	Mal d 1[b]	Recombinant (*E. coli*)
		Thaumatin-like protein	Mal d 2	Apple fruit
		Non-specific lipid transfer protein	Mal d 3	Apple peel
		Profilin	Mal d 4	Recombinant (*E. coli*)

TABLE I. Continued

Source		Protein family/functional properties	IUIS Allergen designation[a]	Origin
		Plant food allergens		
Peach	*Prunus persica*	PR-10	Pru p 1[b]	Recombinant (*E. coli*)
		Non specific lipid transfer protein	Pru p 3[b]	Peach peel
Hazelnut	*Corylus avellana*	PR-10	Cor a 1[b]	Recombinant (*E. coli*)
Hazelnut	*Corylus avellana*	Profilin	Cor a 2	Recombinant (*E. coli*)
		Non-specific lipid transfer protein	Cor a 8[b]	Recombinant *(Pichia pastoris)*
		11S legumin, seed storage globulin	Cor a 9[b]	Hazelnuts
		7S vicillin, seed storage globulin	Cor a 11	Hazelnuts
Peanut	*Arachis hypogae*	7S seed storage globulin	Ara h 1[b]	Peanuts
		2 S albumins	Ara h 2, 6[b]	Peanuts
		11S seed storage globulin	Ara h 3[b]	Peanuts
		PR-10	Ara h 8[b]	Recombinant (*E. coli*)
Celeriac	*Apium graveolens*	PR-10	Api g 1[b]	Recombinant (*E. coli*)
		Profilin	Api g 4	Recombinant (*E. coli*)
		FAD-containing oxidase	Api g 5	Celeriac

[a]Allergen designations as listed in the IUIS Allergen Nomenclature database (www.allergen.org); [b]Allergens already used in routine component resolved diagnosis in either ImmunoCAP or ISAC chip format

TABLE II. Parameters and key methods used for the authentication of purified food allergens

Sequence verification
Matrix assisted laser adsorption MALDI (time of flight (TOF), quadrupole (Q)), *N*-terminal amino acid sequencing
Isoforms
Two dimensional polyacrylamide gel electrophoresis (2D-PAGE)
Folding/structure
Far-UV circular dichroism spectroscopy (CD), 1-D nuclear magnetic resonance spectroscopy (1D-NMR)
Purity/glycosylation/proteolysis
1-D Gel electrophoresis (SDS-PAGE), size exclusion chromatography, electrospray mass spectrometry (ES-MS), capillary electrophoresis
Biological function
Enzymatic activity, ligand binding assay
Immunological activity
IgE-Immunoblot, enzyme linked immunosorbent assay (ELISA), radioallergosorbent assay (RAST), inhibition assay, histamine release assay

Analysis of the 3D-structure of the final purified allergen is necessary, since it affects the immunochemical properties of individual allergens. For *in vitro* diagnosis application, the structural determinants of the purified allergen should be equivalent to the protein present in its natural matrix. Within the EuroPrevall project, far UV circular dichroism was routinely applied to assess the overall secondary structure, and the presence of an alpha helical, a beta-sheet and unordered structures. Furthermore, 1D-nuclear magnetic resonance spectroscopy of the allergens was applied to provide information on the tertiary structure of allergen, discriminating between a rigid 3D-structure, a mobile flexible structure and proteins with features of both, rigid and flexible mobile elements.[6]

Post-translational modifications of proteins may affect their overall allergenic activity. In this respect, glycosylation has an impact on IgE binding activity. For some proteins, glycan moieties contribute to the overall stability of the protein as has been shown for the celery allergen, Api g 5.[7] On the other hand, glycans account for increased recognition of specific IgE antibodies without clinical implications, as is known for some grass pollen allergens. Detection of glycans can be performed by specific staining for lectins. Enzymatic release of glycans with subsequent mass spectrometry analysis is direct proof of glycan moieties.

Finally, the immunological properties of the purified allergen batches have to be determined. The IgE binding properties of a protein are routinely tested in an immunoblot assay or in an ELISA format.[2] While the first approach provides additional qualitative information of the allergen, the second approach enables a semi-quantitative analysis. In addition, the levels of cross reactivity among related proteins can be assessed by inhibition assays in both formats. For *in vivo* approaches, skin prick tests with protein extracts and purified allergens have been performed, inducing a local allergic response in patients. Blood-derived IgE sensitized basophiles can be used for cellular tests. Upon addition of purified allergens, the basophiles are activated due to cross linking of the cell-bound IgE *via* the allergens. As readout of activation of the cells, surface markers such as CD63 or CD203c can be measured by flow cytometry.[8] Alternatively, released histamine is measured by fluorometric detection.

In conclusion, the proof of concept of an allergen library was established within the EuroPrevall framework. Selected allergens from the most important allergenic food sources were purified and characterised applying an array of physico-chemical and immunological methods. During the establishment of the library and collation of the allergen authentication protocols, it became evident, that there is a need for timely and efficient analytical methods for the authentication of proteins. It is expected that the data generated from the EuroPrevall allergen library will contribute to the attainment of important goals, such as the development of allergen standardisation and reference materials that in turn will

improve allergenic risk assessment of food production, diagnosis of food allergy and work towards immunotherapy.

ИЗВОД

УТВРЂИВАЊЕ АУТЕНТИЧНОСТИ АЛЕРГЕНА ХРАНЕ

KARIN HOFFMANN-SOMMERGRUBER

Department of Pathophysiology and Allergy Research, University of Vienna, Austria

Чисти препарати алергена су потребни за тачну, личну дијагнозу алергије на храну. Они се, такође, могу користити за развој осетљивих тестова за детекцију алергена у храни и у имунотерапији. Ови препарати – реагенси морају бити добро окарактерисани и задовољити одређене критеријуме квалитета. У оквиру европског пројекта „EuroPrevall“ развијен је концепт библиотеке алергена у којој се налазе најважнији алергени хране животињског и биљног порекла, уз преглед њихових физичко–хемијских и имунолошких особина. Сарадници са универзитета и из биотехнолошке индустрије су заједнички применили познате лабораторијске технике, као и нове тестове, у анализи најважнијих карактеристика крајњих протеинских препарата. Очекује се да овакав концепт допринесе утврђивању аутентичности алергена, ради рутинске примене у дијагнози и терапији алергија, као и у процени ризика од алергије у производњи хране.

(Примљено 22. октобра 2012, ревидирано 2. јануара 2013)

REFERENCES

1. C. Radauer, M. Bublin, S. Wagner, A. Mari, H. Breiteneder, *J. Allergy Clin. Immunol.* **121** (2008) 847
2. R. Asero, B. K. Ballmer-Weber, K. Beyer, A. Conti, R. Dubakiene, M. Fernandez-Rivas, K. Hoffmann-Sommergruber, J. Lidholm, T. Mustakov, J. N. Oude Elberink, R. S. Pumphrey, P. Stahl Skov, R. van Ree, B. J. Vlieg-Boerstra, R. Hiller, J. O. Hourihane, M. Kowalski, N. G. Papadopoulos, J. M. Wal, E. N. Mills, S. Vieths, *Mol. Nutr. Food Res.* **51** (2007) 135
3. K. Hoffmann-Sommergruber, E. N. Mills, S. Vieths, *Mol. Nutr. Food Res.* **52** Suppl 2 (2008) 159
4. K. Hoffmann-Sommergruber, E. N. Mills, *Anal. Bioanal. Chem.* **395** (2009) 25
5. A. I. Sancho, K. Hoffmann-Sommergruber, S. Alessandri, A. Conti, M. G. Giuffrida, P. Shewry, B. M. Jensen, P. Skov, S. Vieths, *Clin. Exp. Allergy* **40** (2010) 973
6. S. Alessandri, A. Sancho, S. Vieths, C. E. Mills, J. M. Wal, P. R. Shewry, N. Rigby, K. Hoffmann-Sommergruber, *Plos One* **7** (2012) e39785
7. K. Bauermeister, B. K. Ballmer-Weber, M. Bublin, P. Fritsche, K. M. Hanschmann, K. Hoffmann-Sommergruber, J. Lidholm, C. Oberhuber, S. Randow, T. Holzhauser, S. Vieths, *J. Allergy Clin. Immunol.* **124** (2009) 1273
8. S. M. Erdmann, B. Sachs, A. Schmidt, H. F. Merk, O. Scheiner, S. Moll-Slodowy, I. Sauer, R. Kwiecien, B. Maderegger, K. Hoffmann-Sommergruber, *Int. Arch. Allergy Immunol.* **136** (2005) 230.