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Proteomics of allergens

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Abstract

The present state of proteomics research is generally outlined and the character of allergenic compounds briefly elucidated. The principles of experimental approaches to isolation, purification, identification and characterization of allergens and to monitoring of their biological activity are described, with emphasis on the most modern methods. Selected examples are given for illustration and important results are summarized in tables.

Keywords: Proteomics; Allergens

1. Introduction

The enormous recent progress in genomics has boosted experimental attempts at comprehensive description of biological processes through the study of the structure and conformation of proteins expressed on the basis of the genome sequence information, and through description of the mechanism and dynamics of their interactions. The principal problem of proteomics lies in sheer complexity of biological systems in which many macromolecules coexist in fragile dynamic equilibria. Therefore, it is generally necessary to successfully tackle the following tasks:

(a) To obtain a pure component of interest—this primarily depends on efficient, reliable and sufficiently mild separation methods; the time factor may also be important when the dynamics of biological processes are concerned.
(b) To identify the component and elucidate its structure and conformation—this requires a wide spectrum of efficient physical, chemical and biological methods, employing their optimized combinations, hyphenating them, if possible, with the separations (a), and widely utilizing extensive data libraries.
(c) To investigate pertinent interactions of the components, employing combinations of the approaches (a) and (b) and preventing denaturing of the reactants during the experiments.

Allergies belong among very important biological processes and constitute one of serious global medical problems. The most common allergies (type I) are caused by antigens that may initiate an increased production of antibodies from immunoglobulin E (IgE) and are contained in a great variety of natural sources, e.g. plant pollen, animal epithelia, fungi, insect venoms, the faeces of mites, bacteria, or in man-made products, such as foodstuffs and drugs.

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These antigens—allergens—are usually proteins or glycoproteins with molecular masses in a range from ca. 10 000 to 70 000.

The action of allergens is based on their recognition by IgE (or possibly IgG) through linear or conformational epitopes (B cell epitopes); the former contain uninterrupted amino acid sequences, the latter are recognized on the basis of their 3-D structures. So far, no general structural characteristic of allergens has been discovered: linear epitopes differ in the amino acid number and sequence and an exchange of a single amino acid may lead to disappearance of IgE binding, and there is no general conformational pattern discernible [1]. Analogously, glycoprotein allergens, whose carbohydrate moieties consist of only a few monosaccharide units [2], lack any common pattern. For more detailed information, see, Ref. [3] and the special issue of *Journal of Chromatography B* (756:2001), devoted to elucidation of food allergens. Therefore, the efforts at finding some unifying features of allergens and establishing the pertinent structure–function relationships must be based on comprehensive proteomic research as outlined above.

2. A survey of approaches to characterization of allergens and description of their interactions

As pointed out above, this task requires synergic combinations of a great variety of methods, the most important of which are described below. The prerequisite of any kind of study is the isolation of allergens both from the natural sources and the culture lysates in the case of expression in heterologous host. Isolation of allergens from natural material is necessary for further studies with recombinant allergens that can be produced on a larger scale. Therefore, the first step always involves separation procedures permitting isolation of the allergen (a group of isoallergens) from a natural material (a plant, an animal tissue). Then a purification step follows that can be combined on-line with measurements leading to elucidation of the structure and conformation. Finally, measurements are carried out on the pure material in attempts at disclosing the mechanism of the biological effects. Here, the importance of libraries and databases should be stressed, as identification problems cannot otherwise be solved.

2.1. Isolation of allergens from their sources

The first step usually involves the extraction of the parent material, such as a plant or animal tissue or man-made material, with aqueous agents, e.g. water, aqueous buffers (primarily hydrogencarbonate or phosphate), or aqueous salt solutions (e.g. sodium chloride). The extract is subjected to dialysis to remove small molecules and then is lyophilized. A precipitation step with ammonium sulfate is often included. All these procedures should be rapid, to avoid enzymatic degradation of the sample.

The material obtained is subjected to preparative chromatographic procedures, such as size-exclusion and/or ion-exchange chromatography (SEC and IEC, respectively) that may be carried out in a classical or high-performance mode. While the classical chromatography is simple, cheap and has a high sample capacity, the high-performance mode exhibits incomparably better separation efficiency. Affinity chromatography based on specific interactions represents another type of the separation techniques successfully exploited for the allergen isolation (Table 1).

The important alternative is application of immunochemical techniques (Section 2.2.1.) that provide both for isolation and identification/quantification of the substances of interest. Of course, the immune interaction itself must always be assisted by physico-chemical means to attain the goal.

The immunochemical techniques that can be used for isolation of a specific antigen from a heterogeneous mixture involve immunoaffinity chromatography [35] (Table 1) and immunoprecipitation. Immunoprecipitation has a certain advantage over immunoaffinity chromatography in that it requires a smaller amount of serum and provides a higher recovery than the chromatography; however, the results are not sufficiently extensive to permit more specific conclusions on the relative advantages of the two approaches.

2.2. Analytical methods for separation and characterization of allergens

It should again be emphasized that success in elucidating the structure of an allergen and its interactions depends on concerted attacking the

Table 1
Isolation of allergens by means of affinity chromatography

Immobilized ligand	Allergen	Reference
Poly-(L-proline)	Birch profilin[a] and profilactin (*Betula verrucosa*)	[4]
	Recombinant profilins from pear, celery and cherry (*Prunus persica*, *Apium graveoleus*, *Prunus avium*)	[5]
	Profilin from *Mercurialis annua*	[6]
	Recombinant allergen of *Mercurialis annua*	[7]
	Recombinant profilin from sunflower pollen (Hel 2) (*Helianthus annum*)	[8]
	Recombinant profilin from Bermuda grass pollen (*Cynodon dactylon*)	[9]
	Recombinant peanut profilin (*Arachis hypogaea*)	[10]
	Profilin (major allergen) from olive tree pollen (*Oliva europaea*)	[11]
	Celery profilin (*Apium graveoleus*)	[12]
	Profilins from vegetable in general	[13,14]
	Profilins from different sources	[15]
Monoclonal antibody (Mab)	Bermuda grass pollen allergen (*Cynodon dactylon*)	[16,17]
	Olive pollen allergens (*Oliva europaea*)	[18,19]
	English plaintain allergen (*Plantago lancelota*)	[20]
	Isolallergens of *Parietaria judaica* pollen	[21,22]
	Major apple allergen (*Malus domestica*)	[23]
	Parietaria judaica pollen extract	[24,25]
	Peanut major allergen (*Arachis hypogaea*)	[26]
Concanavalin A	Mountain cedar pollen major allergen (*Juniperus ashei*)	[27]
	Mugwort pollen allergen (*Artemisia vulgaris*)	[28]
	Timothy pollen allergen (*Phleum pratense*)	[29]
	Bermuda grass pollen allergen (*Cynodon dactylon*)	[30]
	Grass pollen allergens	[31]
	Olive major pollen allergen (*Olea europaea*)	[32]
Affi-Blue	Bermuda grass pollen allergen (*Cynodon dactylon*)	[33]
	Hypogin from seeds of the peanut (*Arachis hypogaea*) resembling peanut allergen AraHf	[34]

[a] Profilin: panallergen isolated from several different sources.

problem from various angles and thus the methods must be combined ad hoc; therefore, any classification, such as that employed in this review, is purely formal and only serves for making the text sufficiently lucid.

So far, there are the most extensive results on the allergen primary structure: the amino acid sequence has been determined for more than 300 allergens. The secondary structure has also been found for a number of compounds (the beta structure generally predominates). However, the tertiary structure, so important for the antigen–antibody recognition, has only been established for a few substances of interest; there remains very much to be done, primarily using advanced spectroscopy and X-ray diffraction.

2.2.1. Immunochemical methods

Immunochemical methods are based on the binding of an antigen to a specific antibody to form an antigen–antibody complex. The antigenicity of allergens depends on a number of chemical and immunological properties; proteins or glycoproteins should have a molecular mass greater than ca.

10 000, polysaccharides greater than ca. 50 000. Furthermore, allergens must be able to induce an IgE response and mast or basophilic cell granulation [35].

The antigen–antibody interaction can be used for both analytical and preparative purposes. In allergen research, two types of antibody are generally used:

(a) A serum pool containing IgE obtained from the sera of a certain number of patients who exhibit a high sensitivity to the allergen studied
(b) A specific antibody (polyclonal or monoclonal) produced using the pure allergen. A lower specificity of polyclonal antibodies is not necessarily a drawback in comparison with monoclonal ones. However, the allergenic source material consists of a multitude of substances. Here, the hybridoma technique is the first choice to produce monospecific monoclonal antibodies [35,36].

The use of immunochemical techniques for isolation of allergens from a parent material is mentioned in Section 2.1.; their analytical applications fall into three groups.

2.2.1.1. Interaction of immobilized allergenic material with labelled antibody

2.2.1.1.1. *Radio-allergosorbent test* (*RAST*). Radio-allergosorbent test (RAST) [21,37,38] is a diagnostic test for the presence of allergen-specific IgE antibodies and is used for quantification of the total allergenic activity of a preparation. The allergen preparation is usually immobilized on a CNBr-activated paper disk and its interaction with a serum pool labelled with ^{125}I is then evaluated. A similar principle is used in the enzyme-allergosorbent test (EAST), which is an enzyme-based modification of RAST. The serum pool is in this case labelled with an enzyme, usually horseradish peroxidase [23]. Both RAST and EAST are used either as direct binding tests or as inhibition assays [23,39]. These methods serve for quantification of IgE binding to an allergenic source material without differentiating individual allergenic components.

2.2.1.1.2. *Enzyme-linked immunosorbent assay* (*ELISA*). Enzyme-linked immunosorbent assay (ELISA) employs an allergen or an allergenic material immobilized in polystyrene wells where interaction with a labelled antibody occurs. An enzyme (horseradish peroxidase or alkaline phosphatase), or biotinylation (in combination with avidin–horseradish peroxidase) are employed for labelling.

Labelled allergen-specific IgE antibodies make it possible to determine the IgE binding activity of allergenic source materials [39–41] or to characterize recombinant proteins [20]. Monoclonal antibody-based ELISA can be used to quantify allergens, as shown for the allergens Par j 1 [21], Pla 1 1 [20] and Lol p 1 [42].

2.2.1.2. Immunodetection of electrophoretically separated allergenic material with labelled antibody (Western blotting). The immunoblotting (immunodetection) techniques permit identification of allergenic components in the analyzed material after separation of its components by gel electromigration methods.

Sodium dodecylsulfate (SDS) electrophoresis in a polyacrylamide gel (SDS–PAGE) is the most common method for separations of allergenic materials. Isoelectric focusing (IEF) is a method separating protein components under non-denaturing conditions (in contrast to SDS–PAGE). The highest resolution in protein separation is attained by two-dimensional (2D) electrophoresis. In the first dimension, the sample components are separated by IEF and SDS–PAGE is used in the second dimension.

The allergenic components separated in a gel are transferred to a membrane. Various membrane types can be used, nitrocellulose being most common and binding proteins via hydrophobic interactions independent of the protein charge. Positively charged nylon membranes strongly bind negatively charged proteins. Polyvinyldifluoride (PVDF) membranes are recommended because of their mechanical and chemical stability that allows amino acid sequencing of blotted proteins. Electroblotting, used preferably in a semi-dry arrangement, is most often used for protein transfer with high transfer efficiencies.

Membranes with blotted separated protein components are then incubated in the presence of antibodies, which are either labelled, or without a label; in the latter case, the allergen–antibody complex is detected using a labelled secondary antibody. The protein components of the allergenic material are

detected either with IgE (a serum pool of sensitive patients), or with allergen-specific antibodies, especially monoclonal ones. The IgE antibodies are used for the detection of all allergenic components separated by SDS–PAGE (e.g. allergens of plum [43], peach [44], sesame seeds [45], or timothy grass pollen [46]). Analogously, this technique is useful for characterization of recombinant allergens, such as the allergen of *Juniperus* ox pollen r.Jun o 2 [47].

Immunoblotting using specific monoclonal antibodies and human IgE has been employed to elucidate the involvement of the carbohydrate moiety in the allergenicity of Cyn d Bd46K allergen (antibody binding activity before and after periodate treatment) [16].

2.2.1.3. Immunoprecipitation methods. Immunoprecipitation methods are based on the formation of insoluble antigen–antibody complexes. The formation of a precipitate in a gel is evaluated for analytical purposes. Two types of technique are used, either those based only on immunodiffusion, or those involving a combination with electrophoretic methods (rocket immunoelectrophoresis, classical immunoelectrophoresis, crossed immunoelectrophoresis and fused rocket immunoelectrophoresis). Crossed immunoelectrophoresis has been used to study antigenicity of isoallergens from birch [48], *Cocos mucifera* [49] and olive [18] pollens.

2.2.2. Electrophoretic and chromatographic methods

Chromatographic and electrophoretic methods, each involving a number of specialized techniques, should be considered together, as they have many features in common and are complementary in general. This extensive field can be divided, from the point of view of proteomics research, into two characteristic groups of techniques.

2.2.2.1. Gel electrophoresis. Gel electrophoresis is a highly developed approach for characterization of proteins and glycoproteins and thus also of a majority of allergens. Polyacrylamide gel electrophoresis in the presence of SDS (SDS–PAGE) is the commonly used electrophoretic method. The most modern technique for the purpose is two-dimensional (2-D) electrophoresis (IPG–DALT) employing separation according to isoelectric point in a pH gradient in one dimension (IPG strips) and that according to molecular mass in an acrylamide gradient in the other direction. The spots thus separated are typically detected and quantified by image analysis, analyzed either by N-terminal amino acid sequencing or treated with trypsin or endoproteinase and the peptide-containing digest is then analyzed, e.g. by MALDI–TOF mass spectrometry, identifying the components by comparing the results with databases (see, e.g. Ref. [50]). Identification by MALDI–TOF may be difficult when more than two proteins are present in the digest. This problem can be solved by using MALDI–PSD (post-source decay), however, the measurement is then more time-consuming [51]. Allergens in IPG strips may also be identified by immunoblotting with sera of allergy patients and the IgE bound to individual allergens detected using an enzyme- or radiolabelled anti-IgE reagent.

Using narrow, overlapping pH gradients (4.0–5.0, 4.5–5.5, 5.0–6.0, 5.5–6.7, 6.0–9.0) instead of a broad gradient (pH 3–10), a higher resolution may be obtained in the first dimension (e.g. 2286 protein spots compared to 775 spots [52]) Allergens of Orchard grass pollen (*Dactylis glomerata*) were thus studied using the IPG–DALT technique, with four different pH gradients (3.0–10.5, 4.0–9.0, 4.0–7.0, and 6.3–10.5), followed by electroblotting on nitrocellulose membranes and detection employing an allergic patient serum [53].

In the study of allergens it is very important to preserve their native structure, as the epitopes necessary for recognition by IgE are primarily formed by folding of polypeptide chains on the surface of the native protein. To meet this requirement, native 2-D electrophoresis has been developed (see, e.g. Refs. [54,55]), employing CHAPS (3-[3-cholamidopropyl)dimethylammonio]-1-propanesulfonate) as the non-denaturing detergent. The problems associated with a poor solubility of allergens during the separation have been alleviated using tetramethylene sulfone and aminocaproic acid as the solvents [55]).

However, the 2-D gel electrophoresis, whatever highly developed and thoroughly tested, has two drawbacks. First, it has been shown (see, e.g. Ref. [56] and the references therein) that certain classes of proteins are discriminated by this technique. Second, the procedure is rather tedious and time-consuming

and thus studies of the dynamic aspects of immunological interactions may be impaired.

2.2.2.2. High-performance column liquid chromatography (HPLC) and capillary electrophoresis (CE). These two methods complement one another, are marked by high efficiency and provide possibilities of combining them on-line with highly efficient measuring techniques permitting identification of biological macromolecules and elucidation of their structure and conformation.

HPLC is complex, allows many principles of separation, but, on the other hand, may be difficult to optimize and to hyphenate to a sophisticated measuring technique; the last problem is alleviated by the use of capillary HPLC which, however, still fights with technical problems. CE is simpler, but still provides a much more limited range of separation possibilities.

In 1997, the role of HPLC and CE in isolation and characterization of allergens was reviewed [57]. Since that time, there has been a great progress, primarily in the HPLC column technology. Porous materials with large pores and pellicular particles have become common in protein analysis. Monolith columns have enabled fast protein separations due to convective transport through the bed [58]. Proteins with very high molecular masses (exceeding one million) can be analyzed using ultra-short columns, especially with programmed elution. Capillary electrophoresis with fraction collection and subsequent MALDI–TOF characterization of egg allergens has also been reported [59].

Size-exclusion chromatography (SEC) has established itself as the last purification step in the isolation of allergens; it also is capable of providing reliable molecular mass data and thus to complement other molecular mass measurements (e.g. mass spectrometry).

Reversed-phase chromatography (RPC) remains the most versatile technique and is the technique of choice for most separations preceding measurements elucidating the structure (and possibly the conformation) of allergens, such as MS, NMR or X-ray diffraction. A very important derivative of RPC is hydrophobic interaction chromatography (HIC) which employs an increased polarity of the mobile phase by adding salts to it and thus enhancing the ligand–protein interactions; the bound proteins are then eluted with water or dilute buffers.

From the point of view of elucidation of allergens and their interactions, hyphenation of a high-performance technique with a potent measuring technique seems to be the most important. While the combination of high-performance separation techniques with NMR and IR spectroscopy is still not very common, hyphenation to mass spectrometry is commonly used, due to the great recent development in the techniques of interfacing and in the mild ionization (such as electrospray ionization, ESI). The hyphenation of a HPLC or CE separation to a MS or another spectroscopic technique is continuously enhanced by the technical development of capillary high-performance separations.

2.2.3. Methods for determining the structure and conformation of allergens

As can be seen above, the establishing of the structure and conformation of allergens depends on the combination of many approaches, from the

Table 2
Selected recombinant food and pollen allergens

Allergen	Reference
Cherry Pru a 1	[67]
Pru a 2	[68]
Apple Mal d 1	[69]
Celery Api g 1	[66]
Api g 4	[70]
English walnut Jug r 1	[71]
Jug r 2	[72]
Peanut Ara h 1	[63]
Ara h 2	[64]
Ara h 3	[65]
Mustard Sin a1	[79]
Pollen allergens	
Timothy grass Phl p6	[73]
Mercurialis annua Mer a 1	[7]
Short rugweed Amb a 6	[74]
Sunflower Hel a 2	[26]
Bermuda grass Cyn d I2	[37]
Olive tree Ole e 1	[29]
Eastern red cedar Jun o 2	[47]
Mountain cedar Jun a 2	[75]
Cockefoot orchard grass Dac gII	[76]
Birch Bet v 5	[77]
Ragweed Amb a II	[78]

Table 3
Properties of some isolated and characterized allergens

Allergen	MW	AA sequencing	Physicochemical properties	Homology	References
Actimidia chinesis					
Allergen of kiwi fruit (Act c 2)	43 000	N-terminal AA sequence (17 AA)	p*I* 6.9	No homology with Bet v 1 and Mal d 1	[80]
Ambrosia artemisifolia					
Major allergens of short ragweed					
Amb aII (antigen K)	38 000 (3 isoforms)	N-terminal AA sequence	p*I* 5.6–5.85		[78,81]
Amb aI,4 (antigen E)	36 500		p*I* 4.5–5.2		[78]
Allergen Ra 6	11 500 (4 isoforms)	2 N-terminal AA sequence			[82]
r Amb a 6					[74]
Basic antigen AaBA	36 500		p*I* 8.65		[83]
Ragweed allergen Ra3 (AMB A3)		Complete AA sequence (1–101)			[84]
Betula verrucosa					
Birch pollen isoallergens Bet v I, Bet v II		N-terminal AA sequence (51 AA)		N-terminal AA homology to Cor a I	[85]
Birch pollen profilin (Bet v 2)	32 000	Complete AA sequence (1–133)	X-ray crystal structure	Profilin familin	[86]
Castania sativa					
European chestnut pollen allergen Cas s 1	22 000			N-terminal sequence similarity to Bet v 1	[87]
Chamoecyparus obtusa					
Japanese cypress pollen allergen Cha v 1	5000 42 000	N-terminal AA sequence		N-terminal AA sequence identity to Cry j 2	[88]
Cryptomeria japonica					
Japanese cedar pollen allergen Cry j I	45–50 000 (5 isoforms)	N-terminal AA sequence (20 AA identical in all isoforms)	N-linked saccharide chain	N-terminal AA sequence identity to Cha o 1	[89]
Cynodon dactylon					
Bermuda grass pollen allergen					
Cyn d Bd 46 K	46 000	N-terminal blocked internal peptide sequences	Glycoprotein	25–71% identity with cytochrome *c* oxidase	[16]
Cyn d 1	32 000+ 29 000	N-terminal sequence	Glycoprotein	60% homology with Lol p 1	[17,30]
BG 60A	60 000		p*I* 9.7 28% saccharides		[90]
Dactylis glomerata					
Major pollen allergen (Dac g 3)	14 000	N-terminal AA sequence	p*I* 9.0	N-terminal AA sequence identity with Lol p 3	[76]
Festuca pratensis allergen (Fes p 4)	60 000		Glycoprotein, p*I* 8.7–9.1		[91]
Helianthus annus					
Sunflower pollen allergen a,b,c,d	32 000 24 000 55 000				[92]
Juniperus ashei					
Mountain cedar pollen (Jun a 1)		N-terminal AA sequence	Glycoprotein	High degree of homology with Cha o 1 and Cry j 1	[27]

Table 3. Continued

Allergen	MW	AA sequencing	Physicochemical properties	Homology	References
Malus domestica					
Major apple allergen (Mal d 1)	18 000	N-terminal AA sequence			[23]
Mercurialis annua					
Main pollen allergen (Mer a 1)	18 000 14 000				[6]
Olea europaea					
Olive pollen allergen					
Ole e 9	46 400	Complete AA sequence (1–460)			[93]
Ole e 1	19 000+ 17 000 (deglycosylated form)	N-terminal AA sequence (20 AA)	Glycoprotein		[18,32]
Ole e 7	10 000 (polymorphous)	N-terminal AA sequence (21 AA)			[39]
Ole e 4	32 000	N-terminal blocked, AA sequence of internal peptides	p*I* 4.45–5.1	No homology with known proteins	[94]
Ole e 5	16 000	N-terminal AA sequence	p*I* 5.1–6.5	Homology with plant superoxide dismutase	[94]
Parietaria judaica					
pollen isoallergens (Par j 1)	13 000 (1A) + 10 500 (1B)	N terminal AA sequences		Extensive homology between both isoallergens	[22]
Phleum pratense					
Timothy grass pollen allergen (Phl p V)	38 000 32 000	N-terminal AA sequence	p*I* 5.2–7.5 p*I* 4.9–5.9		[46]
Plantago lancelota					
Plantain pollen allergen (Pla l 1)	16–20 000 (isoforms)	N-terminal AA sequence (18 AA)		Partial AA sequence identity with Ole e 1	[40,20]
Prunus persica					
Major allergen of peach (Pru p 1)	9000	Complete AA sequence (1–91)	Non-glycosylated p*I*>9		[44,95]
Prunus armenica					
Major allergen of apricot (Pru ar 3)	9000	Complete AA sequence (1–91)	Hydrophobic cavity	91% sequence identity with peach and almond allergen	[96]
Prunus avium					
Major allergen of cherry (Pru a 1)	18 000	Complete AA sequence (1–160)		59% sequence identity to Bet v 1	[97]
Ricinus communis					
seeds, antigen-5.1	12 000		p*I* 5.1		[98]
Sesamum indicum					
Major allergen of sesam seeds	9000	N-terminal AA sequence	Non-glycosylated p*I* 7.3		[45]

obtaining of a defined material to the final solution of its spatial arrangement. After the isolation of an allergenic fraction and its purification, it is the task of immunological procedures to establish the biological effects, and the task of physico-chemical approaches to define the structure and conformation of the reactants and to say something about the dynamics of the processes involved.

Most allergens are proteins, glycoproteins or peptides. Therefore, to study their interactions and effects, the same methods as those applied to general biologically active macromolecules, should be used.

Consequently, as the structure and conformation are concerned, the solution finally depends on the powerful spectroscopic techniques, such as infrared (IR), Raman and nuclear magnetic resonance (NMR) spectroscopy, and the X-ray diffraction as the most potent method of determining the spatial structure. It is advantageous, if these measuring methods can be connected on-line with the separation technique that is at the top of the sequence producing the pure allergen from the native material. Unfortunately, in contrast to the already common hyphenation of a high-performance separation with MS, the other above methods cannot be as easily combined with separations: IR spectroscopy is hindered by the fact that common mobile phases used in the preceding separation absorb in the IR range and NMR is still difficult and expensive to combine on-line with a separation; Raman spectroscopy is highly promising, but has problems of its own. The method of X-ray spectroscopy cannot be directly connected to a separation method; its main problem in dealing with organic macromolecular substances lies in difficult preparation of measurable crystals of the substances of interest [60]. The advantage of NMR is that it can be performed in solution [61].

2.2.4. Recombinant allergens

The most suitable method for the preparation of large amounts of allergenic proteins is based on molecular biological techniques. cDNA clones have recently been isolated for a number of different types of allergens. The relatively easy production of these molecules enables allergological studies, which are directed to the elucidation of molecular mechanism involved in hypersensitivity reactions [62]. With peanut allergens Ara h 1 [63], Ara h. 2 [64] and Ara H 3 [65], cloned proteins have been subjected to site-directed mutagenesis to modify the IgE-binding epitopes. In most cases, a significant reduction of IgE binding has been achieved. The literature has reported many cases of cloned allergens, which have been subjected to molecular characterization. The use of a recombinant allergen for further studies is dependent on the detailed physico-chemical and biochemical characterization of the proteins purified from natural sources. Recombinant allergens are carefully evaluated for their reactivity with IgE antibodies present in sera from intolerant patients. Natural allergens must be available for comparison with recombinant ones in order to verify the process of molecular expression in heterologous system. Selected recombinant food and pollen allergens are summarized in Table 2. The properties of some isolated and characterized allergens from natural sources (foodstuffs and pollen) are listed in Table 3.

3. Conclusions

As discussed above, the study of biological processes including allergic interactions depends on optimized combinations of physical, chemical and biological approaches. A highest possible technical development is instrumental in the explaining of the character, mechanism and dynamics of the relevant biological processes. The immediate progress in the field of allergy research primarily depends on the cooperation between the physicians (the biological consequences of allergies), the analytical chemists (the isolation, separation and purification of allergens) and the (bio)organic chemists (the establishment of the structure and conformation of the proven allergens).

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