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Structure of allergens and structure based epitope predictions ☆

Fabio Dall'Antonia [a], Tea Pavkov-Keller [b,c], Klaus Zangger [d], Walter Keller [c,*]

[a] European Molecular Biology Laboratory, Hamburg Outstation, Hamburg, Germany
[b] ACIB (Austrian Centre of Industrial Biotechnology), Petersgasse 14, 8010 Graz, Austria
[c] Institute of Molecular Biosciences, University of Graz, Austria
[d] Institute of Chemistry, University of Graz, 8010 Graz, Austria

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ABSTRACT

The structure determination of major allergens is a prerequisite for analyzing surface exposed areas of the allergen and for mapping conformational epitopes. These may be determined by experimental methods including crystallographic and NMR-based approaches or predicted by computational methods. In this review we summarize the existing structural information on allergens and their classification in protein fold families. The currently available allergen-antibody complexes are described and the experimentally obtained epitopes compared. Furthermore we discuss established methods for linear and conformational epitope mapping, putting special emphasis on a recently developed approach, which uses the structural similarity of proteins in combination with the experimental cross-reactivity data for epitope prediction.
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1. Introduction

The three-dimensional structure of clinically relevant allergens is of central importance: (i) It allows the visualization and analysis of surface exposed residues and in combination with experimental or computational methods the actual or putative B-cell epitopes can be elucidated. (ii) Structure can yield information about bound ligands (proteins and/or small molecules), which may modulate the protein's allergenicity. (iii) The allergen structure forms the basis for the rational design of hypoallergenic derivatives, which may be generated through various methods (point mutations, truncations, mosaic proteins, fusion with carrier proteins, etc.).

Most allergens are relatively small, stable and well-structured proteins. Therefore, they are perfectly suited for structural studies by both X-ray crystallography and NMR spectroscopy.

There are also a few examples of obviously unstructured proteins that act as allergens. In particular caseins, which based on NMR and circular dichroism (CD) evidence, are intrinsically unstructured. However, these unstructured allergens might get structured upon interactions with other proteins and/or ligands. In recent years the number of allergen structures deposited in the protein data bank increased exponentially. With the growing number and variety of structures it became clear that there was no "allergen specific fold" emerging. Rather allergens comprised a wide variety of secondary structure compositions and tertiary folds. However, as the structures of many major allergens from representative allergen sources became available it is also becoming clear that most major allergens will be grouped into a limited number of fold and functional families. Here we give an overview of all known allergen structures and their affiliation with known fold families, defined in the PFAM database.

In addition we summarize the techniques used for experimental and computational characterization of conformational epitopes. This part is complemented with an analysis and discussion of the actual knowledge on conformational epitopes gained from the structure of allergen-Fab complexes.

2. Structure determination of allergens

2.1. Crystallographic methods

Type I allergens are proteins of various physicochemical properties and very diverse primary structures and three-dimensional folds. The only common property which has emerged from the characterization of a wide variety of inhalant and food allergens is that the majority exhibits a high solubility in aqueous media. Therefore, allergens may be treated like any soluble protein when it comes to crystallization and crystal optimization, crystallographic data collection, structure solution and refinement. Well established methods exist for all of these steps on the way to the final 3D structure. Here we shall focus on techniques, which are somewhat specific for the structure determination of allergens or

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* Corresponding author. Fax: +43 316 3809897.
E-mail address: walter.keller@uni-graz.at (W. Keller).

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have been applied successfully to important allergens that resisted structure determination in their native form.

Obtaining well diffracting crystals is still the bottleneck of structure determination by crystallography. One of the most important criteria for crystallizability of a protein is the correct fold, its monodispersity and its stability. The method of choice for determining the fold and thermal stability is CD-spectroscopy and its applications to proteins and allergens has been described [1–3]. To increase the solubility of target protein various optimization procedures may be applied (e.g. the sparse matrix approach [4], additive and detergent screens) and in combination with the Thermofluor method [5] they allow for the parallel screening of vastly different conditions. In cases where the allergen is highly soluble and well ordered, but still refuses to crystallize, flexible ends or linkers between ordered domains can be present, as for the case of Phl p 5. Here it will be necessary to determine the flexible regions by experimental (e.g., limited digests) or computational methods [6,7]. Alternatively, point-mutants with changes of surface exposed residues may be necessary to promote beneficial crystal packing interactions [8]. After engineering the protein to remove the flexible parts either the truncated full-length allergen or its folded domains are submitted to crystallization.

A quite different approach which has been applied to the structure determination of allergens is the use of a fusion chaperon, where the smaller allergen is fused to a larger fusion partner, which has been shown (or even optimized) to promote crystallization [6]. This approach was successful for the structure determination of two allergens: Der p 7 and Ara h 2 (Fig. 1) [9]-[10]. Finally, the use of a specific binding partner (e.g., Fab or Fv) for complex formation can also enhance the crystallizability of the allergen – the Fab acts as a non-covalent crystallization chaperon. In addition the complex structure yields the exact information about the binding site (discussed in detail in Section 4.2).

2.2. NMR methods

2.2.1. Preparation of protein

The very first NMR studies on allergen structures were carried out using proteins isolated from natural sources. In particular, the ragweed allergen Amb t 5 (previously called Ra5 and Amb t V) structure was determined using homonuclear experiments on isolated protein at natural isotopic abundance [11–13]. Because of the large protein amounts needed and to enable isotopic enrichment, recombinant proteins were used for most other NMR studies of allergens. Therefore, the cDNA of the allergen or a synthetic DNA corresponding to the desired protein is cloned typically into overexpression vectors of *Escherichia coli* cells. While rich media (e.g., LB broth) can be used for protein expression at natural abundance, minimal medium containing $^{15}NH_4Cl$ as nitrogen and ^{13}C–glucose as carbon source are typically used.

2.2.2. NMR assignment and structure determination

2.2.2.1. At natural abundance. Due to the limited spectral dispersion of ^{1}H NMR spectra, structural protein NMR studies on allergens at natural isotopic abundance are limited in size to $<\sim 15$ kDa. Chemical shift assignment of ^{1}H nuclei (protons) is achieved by first identifying spin systems of individual amino acids in a 2D TOCSY spectrum and subsequently establishing sequential connections via short through-space proton-proton distances (NOEs or Nuclear Overhauser Enhancements) [14]. This approach has been employed to obtain the structures of Amb t V (5 kDa) [11] and Phl p 2 (11 kDa) [15]. Due to the low spectral resolution of ^{1}H and ambiguities in using NOEs for sequential assignment nowadays almost all proteins used for NMR structural studies are labeled with stable isotopes to circumvent these difficulties.

2.2.2.2. Using isotopically enriched protein. The use of proteins enriched with ^{15}N and ^{13}C allows the use of these additional NMR active isotopes in the assignment and structure determination approach. Both nuclei offer a much better spectral resolution and relaxation behavior (narrower line-width) than protons and the direct connectivities by chemical bonds allows the signal assignment to proceed via through-bond (scalar couplings) rather than sometimes ambiguous through-space proton-proton distances (NOEs). ^{1}H, ^{15}N and ^{13}C resonances can be assigned using standard 3D triple-resonance experiments, which allow the sequential walk along the backbone by connecting the chemical shifts of backbone amide N and H, Cα, Cβ and C′ of a certain amino acid (i) with the corresponding frequencies of its two sequential neighbours ($i-1$ and $i+1$) [16]. Side-chain proton and carbon assignment is then achieved using experiments that correlate them to the previously assigned backbone nuclei using e.g., HCCONH, CCONH and HCCH TOCSY spectra. Once almost complete ^{1}H, ^{13}C and ^{15}N assignment is accomplished structural restraints need to be acquired. As for the vast majority of proteins, also for allergens these have been mainly NOEs, but also dihedral angle restraints obtained from three-bond coupling constants and for some more recent studies orientational restraints from dipolar couplings in weakly aligned media [17]. For an accurate 3D structure determination a large number of restraints (>1000 for a protein of 10 kDa or above) is needed. Due to increasing line-width and number of signals at higher molecular weights a complete atomic-resolution 3D structure determination by NMR spectroscopy faces a size-limit which is currently around 40 kDa. For large proteins (>30 kDa) the use of TROSY-type (Transverse Relaxation Optimized Spectroscopy) [18] experiments is preferred which results in narrower lines and higher intensities.

2.2.3. Information about protein dynamics

One of the advantages of NMR spectroscopy is that in addition to the structure also the dynamical behaviour of a protein can be investigated. In particular, backbone amide ^{15}N T_1 and T_2

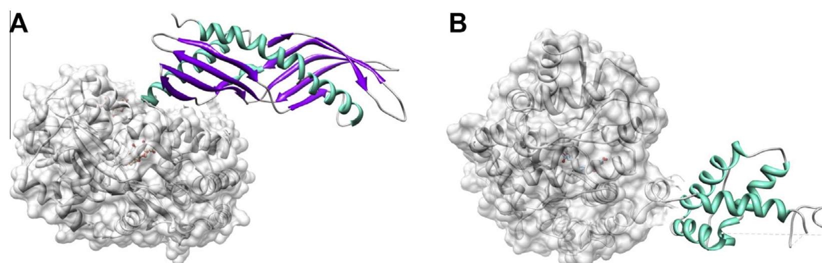

Fig. 1. MBP-fusions act as crystallization chaperons. (A) Der p 7 (PDB: 3h4z) and (B) Ara h 2 (3OB4) are shown as maltose-binding protein (MBP)-fusion proteins. Allergen structures are shown in ribbon representation and are colored according their secondary structure composition (α-helices in cyan, β-sheets in magenta). MBP is additionally shown as surface representation (gray). Dashed lines indicate the disordered regions that are missing in the crystal structures.

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