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Structure of the house dust mite allergen Der f 2: Implications for function and molecular basis of IgE cross-reactivity

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Abstract The X-ray structure of the group 2 major allergen from *Dermatophagoides farinae* (Der f 2) was determined to 1.83 Å resolution. The overall Der f 2 structure comprises a single domain of immunoglobulin fold with two anti-parallel β-sheets. A large hydrophobic cavity is formed in the interior of Der f 2. Structural comparisons to distantly related proteins suggest a role in lipid binding. Immunoglobulin E (IgE) cross-reactivity between group 2 house dust mite major allergens can be explained by conserved surface areas representing IgE binding epitopes.

Keywords: *Dermatophagoides farinae* group 2 major allergen; Cross-reacting epitope; Hydrophobic cavity; Lipid binding

1. Introduction

Inhalation allergy to house dust mites is among the most widespread allergic diseases, particularly in the Western hemisphere and causes symptoms typical for type 1 immediate hyperreactivity, such as rhinocunjunctivitis and asthma. The most prevalent house dust mite species belong to the genus *Dermatophagoides*, i.e., the European house dust mite, *D. pteronyssinus*, and the American house dust mite, *D. farinae*, but other species, such as *Euroglyphus maynei* are also considerably distributed. Due to the taxonomical relationship house dust mites from different species contain homologous allergens, which cause immunoglobulin E (IgE) cross-reactivity [1,2]. The most important allergens in terms of prevalence of reactivity are the group 1, 2, 3 and 9 allergens, to which more than 90% of mite allergic patients have IgE. Group 1 and group 2, however, seem to be most important since they account for most of the IgE on a quantitative basis. Group 2 allergens, including major type 2 allergen from *Dermatophagoides farinae* (Der f 2), are 129 amino acid proteins with a molecular weight around 15 kDa. Their biological functions are not known, but sequence analyses has recently indicated that they contain the MD-2-related lipid-recognition (ML) domain [3]. Phylogenetic analysis of ML domain proteins grouped seven class 2 mite allergens with the human epidymal secretory protein (HE1; also called NPC2) that is believed to be involved in cholesterol transport [4]. The structure of HE1 has recently been determined [5] and HE1 was shown to bind the cholesterol analog dehydroergosterol with submicromolar affinity at both acidic and neutral pH.

The structure of Der f 2 has previously been investigated by NMR spectroscopy [6] and the homologous allergen, major type 2 allergen from *Dermatophagoides pteronyssinus* (Der p 2) (87% sequence identity), has been studied by both NMR [7] and X-ray crystallography [8] yielding similar structures. The NMR structure of Der f 2, however, is quite different from that of Der p 2. A crystallization note on Der f 2 has been published recently [9], but so far no crystal structure has been deposited in the PDB database.

The X-ray structure determination of Der p 2 showed additional electron density that was suggested to originate from an unidentified lipid ligand, in agreement with its similarity to HE1. The Der f 2 structure provides further insight into the lipid-binding properties of house dust mite group 2 allergens.

It is well established that treatment of allergic individuals with allergen vaccines in controlled doses has curative potential; this treatment is referred to as allergy vaccination or specific immunotherapy. An inherent aspect of allergy vaccination is a small but significant risk of inducing serious side effects, such as anaphylactic shock. Several alternatives to the traditional injection therapy are currently under investigation, for example sublingual administration or use of recombinant allergens [10]. Also, concepts for the modification of recombinant allergens to reduce allergenicity are actively being pursued [11]. Rational modification of Der f 2 requires high-resolution knowledge of the molecular structure of the allergen. The present study provides this structural basis for Der f 2 along with analysis of its cross-reacting epitopes.

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Abbreviations: Eur m 2, *Euroglyphus maynei* major allergen 2; GM2-AP, GM2-activator protein; HE1, human epidymal secretory protein; IgE, immunoglobulin E; Lep d 2, major type 2 allergen *Lepidoglyphus destructor*; Der f 2, major type 2 allergen from *Dermatophagoides farinae*; Der p 2, major type 2 allergen from *Dermatophagoides pteronyssinus*; ML, MD-2-related lipid-recognition; MR, molecular replacement; PEG, Polyethylene glycol; RhoGDI, Rho-specific guanine dissociation inhibitor; RMSD, root mean square deviation

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2. Materials and methods

2.1. Expression and purification

cDNA coding for Der f 2 (provided by Heska Corporation, Fort Collins, CO) was cloned into the expression vector, pGAPZα-A (Invitrogen) and expressed in the yeast, *Pichia pastoris*. Der f 2 was expressed as a fusion to the α-factor signal sequence from *Saccharomyces cerevisiae*. The signal peptide was cleaved off by the constitutively expressed Kex 2 proteinase, leading to the secretion of mature Der f 2 into the culture supernatant. For purification of recombinant Der f 2, a 1 L culture was grown in a 5 L culture flask at 30 °C using an orbital shaker at 225 rpm. Culture supernatants expressing recombinant Der f 2 were harvested after 72 h and precipitated sequentially with ammonium sulfate (0–50%, 50–80%). The precipitate was dissolved in 10 mM MES, pH 6.0 and desalted using a fast desalting HR 10/10 column and partly purified using a Mono S HR 5/5 column. The Der f 2 was further purified by gel filtration on a Superdex 75 column in 10 mM NH_4HCO_3, pH 6.0 followed by a Mono Q HR 5/5 column and concentrated.

2.2. Crystallization

Crystallization was carried out at 20 °C using the hanging-drop vapor diffusion method. Der f 2 crystals were obtained by mixing 2.5 μL protein solution at 1.6 mg/mL with 2.5 μL reservoir solution (30%, w/v, polyethylene glycol (PEG) 4000, 0.1 M sodium citrate, pH 6.0, 0.2 M ammonium acetate).

2.3. Data collection and processing

Crystals of Der f 2 were flash-cooled to 100 K and data were collected in-house using a Rigaku RU300 rotating anode (Rigaku Instruments, Tokyo, Japan) equipped with a MAR345 image plate detector. Diffraction data were processed with the CCP4 programs MOSFLM and SCALA [12]. All statistics are listed in Table 1.

2.4. Structure determination and refinement

The Der f 2 structure was determined by the molecular replacement method using the program Phaser from CCP4 [10]. A crystal structure of Der p 2 ([8]; PDB code 1ktj) was used as the search model. A solution comprising two molecules was obtained. Subsequently, automated model building was performed with the program ARP/wARP [13]. This resulted in the tracing of 84% of the residues. The missing residues were inserted by manual model building in the program O [14]. Initially, refinement of the structure was performed using the program REFMAC 5 [15]. In the final rounds, the program CNS was applied [16]. Water molecules and two PEG were gradually introduced into the structures. The quality of the model was analyzed using the program PROCHECK [17]. Figures were prepared using the program Pymol [18].

Table 1
Crystal data, data collection, and refinement statistics

Space group	*P*1
Unit cell parameters (Å, °)	$a = 25.7$, $b = 47.6$, $c = 48.3$, $\alpha = 86.0$, $\beta = 75.9$, $\gamma = 82.8$
Molecules (a.u.)	2
Resolution range (Å)[a]	24.4–1.83 [1.93–1.83]
Unique reflections	18 043
Average redundancy	3.9
Completeness (%)	93.6 [89.7]
R_{merge} (%)	4.9 [24.0]
$I/\sigma(I)$	9.0 [3.0]
Non-hydrogen atoms	2277
Amino acid residues	258
Water molecules	240
R_{cryst} (%)[b]	22.2
$R_{free,\ 5\%}$ (%)[c]	25.7
RMS bond lengths (Å)/angles (°)	0.008/1.7
Residues in allowed regions of Ramachandran plot (%)[d]	97.5
Average B values (Å^2) for protein/PEG/water atoms	28.2/49.8/35.1

[a]The values in brackets correspond to the outermost resolution shell.
[b]$R_{cryst} = \sum_{hkl}(||F_{o,hkl}| - |F_{c,hkl}||)/|F_{o,hkl}|$, where $|F_{o,hkl}|$ and $|F_{c,hkl}|$ are the observed and calculated structure factor amplitudes.
[c]R_{free} is equivalent to the R-factor, but calculated with reflections omitted from the refinement process (5% of reflections omitted).
[d]The Ramachandran plot was calculated according to Kleywegt and Jones [30].

2.5. Analysis of cross-reacting epitopes

Buried side-chains were identified by calculating the side-chain depth using a procedure modified from the Biopython package [19]. The side-chain depths and the corresponding residue solvent accessible surface areas were calculated using CCP4 program AREAIMOL [10].

Protein data bank accession number. The atomic coordinates and structure factors of Der f 2 have been deposited with the RCSB Protein Data Bank under the accession code 1XWV.

3. Results and discussion

3.1. The crystal structure of Der f 2

Two very similar molecules (molecule A and B) are observed in the asymmetric unit of the crystal (root mean square deviation (RMSD) on 129 Cα atoms equals 1.0 Å). Molecule B is less well defined and has higher B values (B_{avg} = 30 Å^2) than molecule A (B_{avg} = 23 Å^2) (Fig. 1A and B). The overall Der f 2 structure comprises a single domain of immunoglobulin fold with two anti-parallel β-sheets overlying each other. One β-sheet consists of five β-strands of residues 6–7 (A′), 51–58 (C), 61–63 (C′), 104–112 (F) and residues 115–122 (G). The other β-sheet contains three β-strands: residues 13–18 (A), 34–42 (B) and 85–93 (E). Der f 2 has two additional short β-strands B′ (residues 27–30) and G′ (residues 125–128). Both molecules show flexibility in the large loop (residues 64–84) between strand C′ and strand E (Fig. 1A and B). Three disulfide bonds are present between cysteine residues 8–119, 21–27 and 73–78. This disulfide arrangement is different from what is normally found in immunoglobulin-fold domains with only a single disulfide bond.

The structure of Der f 2 is very similar to the crystal structure of Der p 2 [8]. The three disulfide bonds are conserved and superimposition of all Cα atoms in Der f 2 and Der p 2 shows a RMSD of 0.51 Å (molecule A) and 1.20 Å (molecule B), respectively. The NMR structure of Der f 2 based on 1086 NOE restraints [6] displays the same overall fold, however, the RMSD on Cα atoms of the average Der f 2 NMR structure and the crystal structure of Der f 2 (molecule A) is 5.52 Å. This difference is perhaps due to the experimental conditions used in the NMR experiment (55 °C, 140 mM *N*-octyl-β-D-glucoside).

3.2. Central hydrophobic cavity suggests a role in lipid binding

In the interior of the Der f 2 structure, a large cavity is formed almost exclusively of hydrophobic and aromatic residues (Fig. 1C). The cavity is ~10 Å wide, ~20 Å long and ~4 Å deep with a depression created by the side chains of residues Leu37 and Val108 that are located on opposite sides of the cavity. In this hydrophobic cavity, an elongated U-shaped electron density is observed (Fig. 1A and B). We have modeled one PEG fragment into this density in molecule A and B, respectively, as the crystals were grown in PEG4000. Well-defined electron density for the PEG molecule is seen in molecule A, whereas discontinuous density is found in molecule B. A narrow tunnel leads to the exterior of the protein. The tunnel

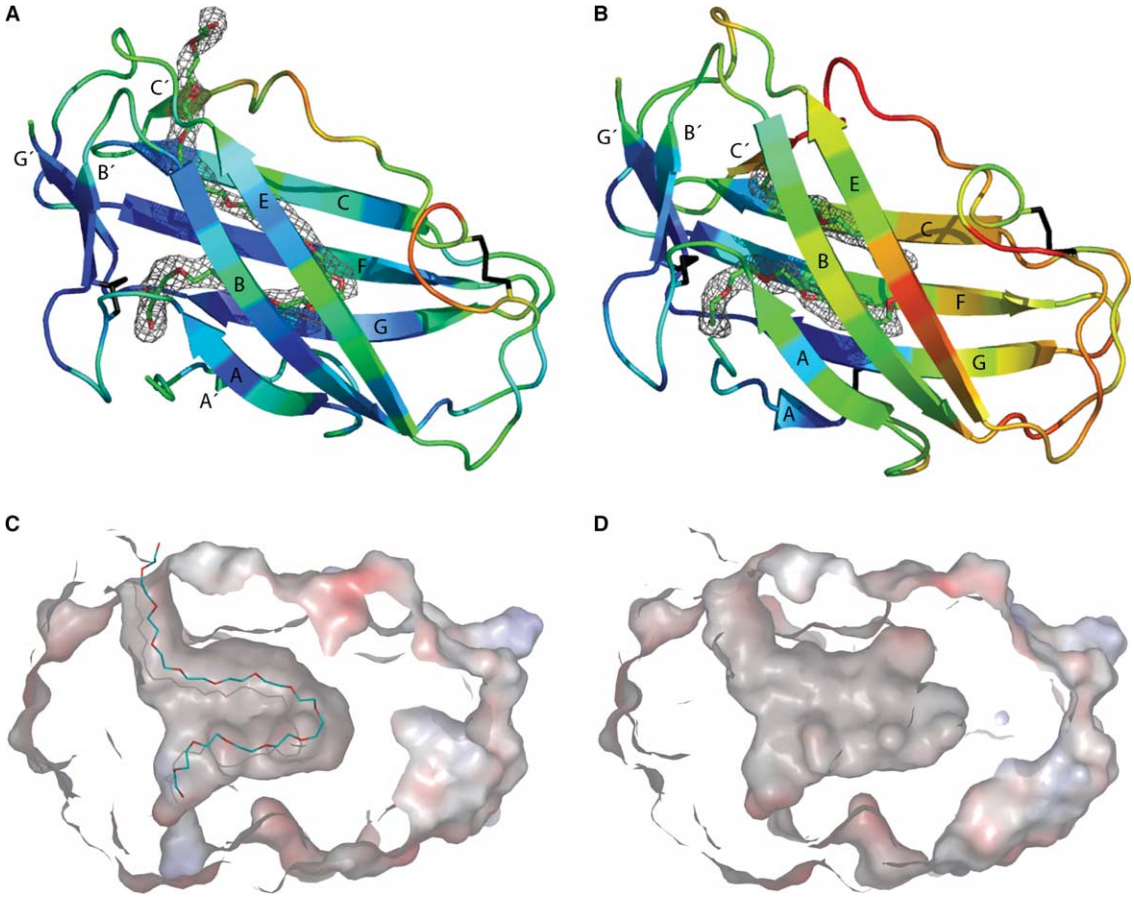

Fig. 1. (A, B) Cartoon representation of the overall structure of the two Der f 2 molecules in the asymmetric unit of the crystal coloured by *B* values (blue is low and red is high values, ranging from 12 to 50 Å^2). The three disulfide bonds are colored black. $2F_o - F_c$ electron density in the central cavity is shown (in gray) and contoured at 1.0σ. A PEG molecule was modeled into this density. The structural elements were labeled according to the Der p 2 structure [8]. (C, D) Solvent accesible surface areas of the molecules (C) Der f 2 with a PEG molecule and (D) Der p 2. The surface is slapped in order to reveal the central cavity. The surfaces are colored after electrostatic potential (red is −10 kT and blue is 10 kT).

opening is approximately 3 Å in diameter and is formed by residues Leu58, Ile63, Pro66, Trp92, Pro95, Ile97 and Pro99. The size of this cavity entrance is large enough for a PEG molecule or a natural hydrophobic ligand to enter. The other end of the PEG molecule also protrudes at the surface on the other side of the molecule; however, the opening here is only about 1 Å wide.

Like the overall fold of Der f 2, the cavity of the protein resembles that of Der p 2 (Fig. 1C and D). For Der p 2, this internal cavity was described containing an unidentified hydrophobic ligand independent of the presence of PEG [8]. Only four residues facing the cavity differ in the two proteins; residues Leu58, Phe75, Ala88 and Leu110 in Der f 2 correspond to Ile, Tyr, Ile and Val in the Der p 2 structure. The NMR structures of Der p 2 and Der f 2 do not show this kind of cavity, here the distances between the β-sheets are shorter. This discrepancy could be a consequence of the different methods employed or it could indicate the presence of an ''open'' and a ''closed'' state.

The high sequence and structural similarity between the two allergens indicate that the proteins have similar functions in the two mite species. The biological function of Der f 2 and Der p 2 is not known, but they show distant homology to several lipid-binding proteins, the ML family of proteins. The ML family proteins consists of approximately 150 residues, have two pairs of conserved cysteine residues and are probably secreted. Among these lipid-binding proteins is the human Rho-specific guanine dissociation inhibitor, RhoGDI [20,21], the mammalian secretory protein HE1 known to bind cholesterol [5,22], MD-2 that binds to lipopolysaccharides [23] and GM2-activator protein (GM2-AP) crystallized with two lipids and a fatty acid [24]. The ML protein family comprises members that are involved in lipopolysaccharide signaling, lipid recognition and lipid metabolism, but many members as, e.g., Der f 2 and other allergens are of unknown function [3]. The structural similarity and the fact that many of the ML family proteins also have a similar hydrophobic cavity and are known to bind lipids strongly suggest a natural lipid ligand of the Der f 2 molecule.

3.3. Epitopes and cross-reactivity

The human allergic symptoms are triggered by allergen mediated cross-linking of IgE molecules attached to FcεRI-receptors on the surface of mast cells [25]. Consequently, amino acid residues located on the molecular surface of the allergen form epitopes that mediate interactions with IgE antibodies. IgE cross-reactivity is observed when similar epitopes on different allergens bind the same IgE molecule with similar affinity.

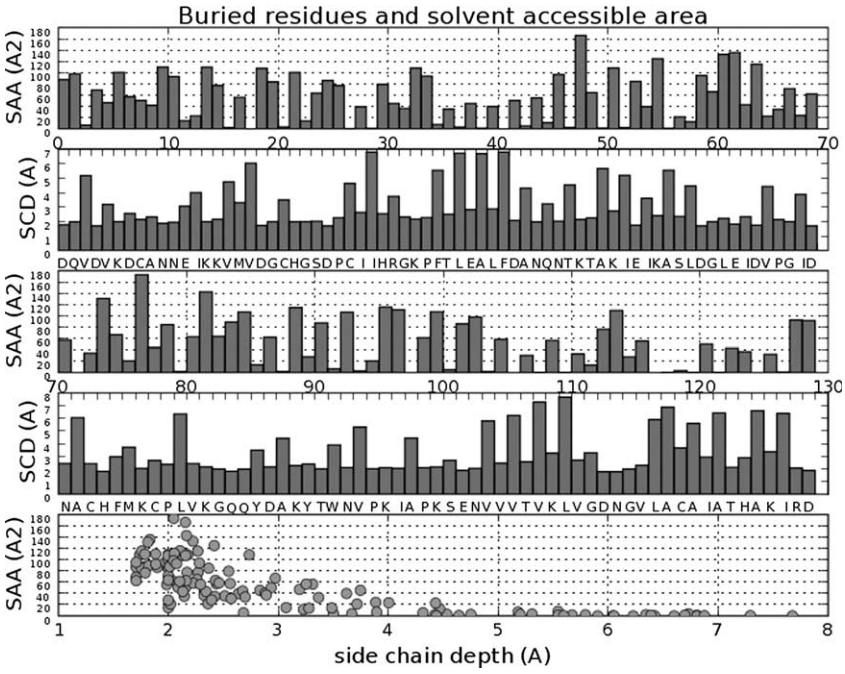

Fig. 2. Solvent accesible surface areas and cross-reactivity. Plots of solvent accessible area (SAA, Å^2) and side-chain depths (SCD, Å) for Der f 2. The lowest panel shows the expected reciprocal correlation of SAA and SCD.

For Der f 2 amino acids on the exterior surface were identified by their contributions to the solvent accessible surface per residue and the side chain depth (i.e., the average distance from the side chain atoms to the solvent accessible surface) (Fig. 2). These measures clearly identify the 45 residues situated in the interior of the protein, and hence, the remaining 84 side chains are identified as located on the molecular surface and potentially epitope forming.

IgE cross-reactivity between natural Der f 2 and Der p 2 is well established [2]. Fig. 3A shows the molecular surface of Der f 2 colored according to amino acid conservation with the homologous allergen Der p 2. It is evident that the majority of the surface residues are conserved leading to very similar epitopes forming the structural basis of cross-reactivity between the two house dust mite allergens.

IgE cross-reactivity between both allergens and the house dust mite *Euroglyphus maynei* major allergen (Eur m 2), has been observed by antibody binding studies using recombinant isoallergens [1]. Further non-pyroglyphid storage mites, such as *Lepidoglyphus destructor* (Lep d 2), *Glycyphagus domesticus* and *Tyrophagus putresscentiae* have been reported to cause occupational allergy in farmers and bakers [26,27], but studies of IgE cross-reactivity between group 2 allergens from these mites and house dust mite group 2 allergens have yielded conflicting results [1,28]. According to a surface comparison of Der f 2 and Lep d 2, see Fig. 3B, conserved areas are scattered and limited in size supporting the absence of cross-reactivity. However, recent studies of a Bet v 1 mutated recombinant allergen have indicated that IgE from individual patients react to a rather limited section of the molecular surface, which differs from patient to patient [29]. An explanation for the conflicting results obtained by serological analysis of cross-reactivity between group 2 allergens from house dust mites and storage mites could therefore be a dependence on epitope repertoires defined by individual patients.

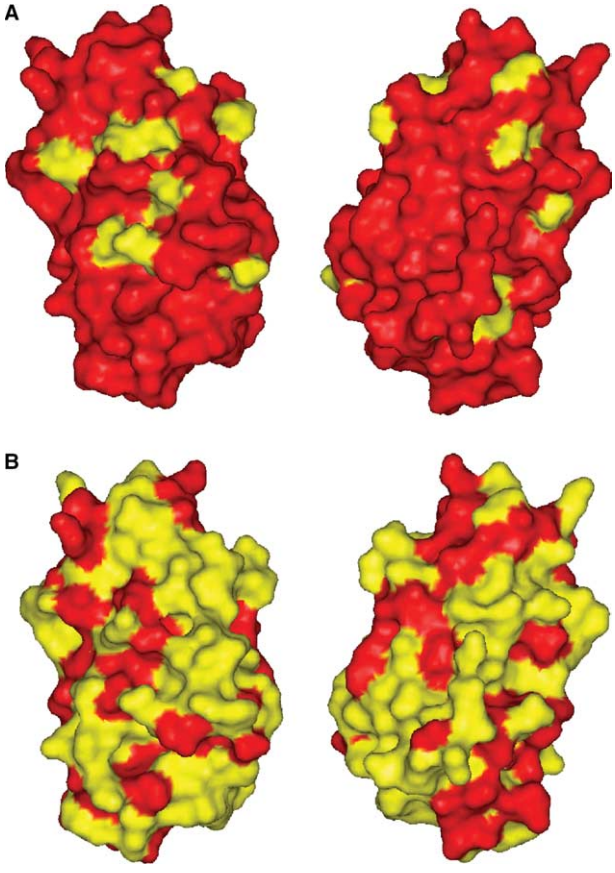

Fig. 3. Molecular surfaces of Der f 2 colored after amino acid conservation compared with the closely related (A) Der p 2 and the distantly related (B) Lep d 2. Red indicates identical and yellow different amino acids; right figures are turned 180°. See text for details.

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