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# Crystallization and preliminary X-ray analysis of Der f 2, a potent allergen derived from the house dust mite (*Dermatophagoides farinae*)

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Although a number of allergens have been identified and isolated, the underlying molecular basis for the potent immune response is poorly understood. House dust mites (*Dermatophagoides* sp.) are ubiquitous contributors to atopy in developed countries. The rhinitis, dermatitis and asthma associated with allergic reactions to these arthropods are frequently caused by relatively small (125–129 amino acids) mite proteins of unknown biological function. Der f 2, a major allergen from the mite *D. farinae*, has been recombinantly expressed, characterized and crystallized. The crystals belong to the tetragonal space group $I4_122$, with unit-cell parameters $a = b = 95.2$, $c = 103.3$ Å. An essentially complete (97.2%) data set has been collected to 2.4 Å at a synchrotron source. Attempts to solve the crystal structure of Der f 2 by molecular replacement using the NMR coordinates for either Der f 2 or Der p 2 (the homologous protein from *D. pteronyssinus*) failed, but preliminary searches using the crystalline Der p 2 atomic coordinates appear to be promising.

## 1. Introduction

The global incidence of atopic diseases such as asthma and dermatitis is rapidly increasing (Marshall & Davis, 1997). Despite the commitment of immense pharmaceutical resources and the efforts of a dedicated medical sub-speciality, allergic reactions continue to cause substantial morbidity and mortality. Avoidance or desensitization to a specific allergen is possible, but in many cases the offending agent cannot be identified or adequately removed from the environment.

Allergic reactions appear to be initiated by the specific binding of allergens to immunoglobulin E (IgE) antibodies that cross-link the high-affinity IgE receptors on mast cells and basophils. The three-dimensional structures of several pollen allergens have been determined, including the major ragweed allergens Amb t 5 and Amb a 5, the major birch allergen Bet v 1 and a minor birch allergen, profilin. The structural basis for the exquisite immune response elicited by these proteins from susceptible hosts remains unclear, but is of keen biomedical and pharmaceutical interest (Furmonaviciene & Shakib, 2001).

Two common house mites, *Dermatophagoides farinae* and *D. pteronyssinus*, are recognized as the primary source of house dust allergens. On the basis of skin tests, 75–80% of allergic asthmatics display mite sensitivity. Numerous proteins derived from these arthropods have been isolated and characterized. The major allergens are divided into two general groups based on molecular properties. Group 1 allergens are cysteine proteases with a common molecular weight of 25 kDa. Group 2 allergen proteins, including Der f 2, are small 129 amino-acid proteins (14–15 kDa) of undefined biological function. Homologous proteins are found in the human epididymis (HE1), bovine milk (EPV20) and in the moth *Manduca sexta* trachea (esr16). Group 2 allergens bind to the surfaces of bacteria and may be important to mite immune function (Ichikawa *et al.*, 1998).

The NMR analysis of Der f 2 revealed a single-domain protein with an s-type immunoglobulin fold and modest sequence/structural homology with the third and fourth domains of human blood coagulation factor XIII. Surprisingly, the NMR structure of the highly homologous (87% sequence identity) Der 2 antigen from *D. pteronyssinus* (Der p 2) was substantially different to that of Der f 2 (Mueller *et al.*, 1998, 2001). The poor three-dimensional alignment (4.85 Å r.m.s.d.; *Z* score 8.58) was ascribed to differences in solution conditions during data collection.

The 2.15 Å crystallographically derived structure of Der p 2 is not highly homologous with canonical Ig-like proteins, but more closely resembles that of the human Rho-specific guanine dissociation inhibitor (RhoGDI). The two $\beta$-sheets of the crystalline Der p 2 form an internal hydrophobic cavity that is absent in the NMR models (Derewenda *et al.*, 2002). Indistinct non-assigned electron density within the cavity is compatible with the

presence of two presumably hydrophobic ligands. This intriguing finding suggests that lipids or lipid binding may be important to the function of Der f 2 or to its potency as an allergen. Although the three-dimensional structure of Der f 2 is likely to closely resemble that of Der p 2, small perturbations in structure may have important biological and immunotherapeutic consequences. Hopefully, the putative hydrophobic ligands, if present in Der f 2, will prove to be more readily interpretable.

The refined three-dimensional structure of Der f 2 will serve as a useful complement to ongoing genetic analyses (Takai *et al.*, 1997, 2001; Takai, Ichikawa, Hatanaka *et al.*, 2000; Takai, Ichikawa, Yokota *et al.*, 2000; Korematsu *et al.*, 2000). Improving the ability of recombinant allergens to desensitize patients, while reducing the risk of anaphylaxis, should greatly improve the effectiveness and safety of immunotherapy (Valenta, 2002). Experimental disruption of the disulfide bond that links the N- and C-termini of Der f 2 has already demonstrated that the binding of an allergen (Der f 2) to IgE can be separated from its associated interactions with the T-cell (Takai *et al.*, 1997).

## 2. Materials and methods

Der f 2 protein was expressed and purified from *Escherichia coli* (Nishiyama *et al.*, 1995). Crystallization of Der f 2 protein was carried out using the hanging-drop method of vapour diffusion and a sparse-matrix strategy (McPherson, 1999). Der f 2 crystals were obtained by mixing 2.0 µl of protein with 2.0 µl of reservoir solution in the hanging drop. The protein-stock concentration was 26 mg ml$^{-1}$ and the reservoir contained 100 m*M* Tris pH 8.0, 2.0 *M* sodium sulfate. Crystals appeared in 2–3 d and grew to dimensions of 0.4 × 0.5 × 0.4 mm after 7 d (Fig. 1). Initial trials with ammonium sulfate yielded large prismatic crystals that were poorly ordered. The diffraction properties of Der f 2 crystals were markedly improved by the substitution of sodium sulfate.

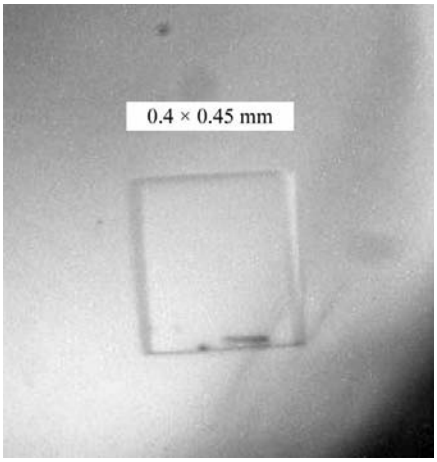


**Figure 1**
Single crystal of Der f 2, a major allergen from the house mite *D. farinae*.

Single crystals suspended in native liquor were trapped in a mounting loop and dipped briefly into a cryoprotectant solution (30% glycerol, 100 m*M* Tris pH 8.0, 2.0 *M* sodium sulfate) before flash-cooling in a cold nitrogen-gas stream. The flash-cooling process did not appear to significantly affect either the resolution limits or the mosaicity of the diffraction data compared with room-temperature images. Data collection was performed using a MAR CCD imaging-plate detector installed on beamline 7-1 at the Stanford Synchrotron Radiation Laboratory. A total of 158° of data (1° per frame) were collected and processed using the *HKL* program package (Otwinowski & Minor, 1997).

## 3. Results

Based on data-refinement statistics and systematic absences, Der f 2 crystallizes in the space group $I4_122$, with unit-cell parameters $a = b = 95.2$, $c = 103.3$ Å. The data set collected from two crystals included 271 420 measurements, which were reduced in a single refinement run to 9107 unique reflections at 2.4 Å. The merged $R_{sym}$ of 5.5% was not substantially different to that obtained for single crystals (Table 1).

**Table 1**
Data-processing statistics for Der f 2.

Values in parentheses refer to the highest resolution data shell.

| | |
|---|---|
| No. of images (1° per image) | 158 |
| No. of crystals | 2 |
| Space group | $I4_122$ |
| Unit-cell parameters (Å) | $a = b = 95.2$, $c = 103.3$ |
| Mosaicity (°) | 1.525 |
| Resolution range (Å) | 30–2.40 (2.44–2.40) |
| No. of measured reflections | 271420 |
| No. of measured unique reflections | 9107 |
| $R_{sym}$† (%) | 5.5 (22.0) |
| Completeness (%) | 97.2 (92.2) |
| $I/\sigma\langle I\rangle$ | 17.7 (2.7) |

† $R_{sym} = \sum|I - \langle I\rangle|/\sum I$, where $I$ is the observed intensity and $\langle I\rangle$ is the average intensity derived from multiple observations of symmetry-related reflections.

Attempts to solve the crystal structure of Der f 2 by molecular-replacement searching with the NMR coordinates for Der f 2 (Ichikawa *et al.*, 1998: PDB codes 1ahk, 1ahm) or Der p 2 (Mueller *et al.*, 1998: PDB code 1a9v) failed. An unambiguous peak could not be identified from the *AMoRe* (Navaza, 1994) rotation function despite the use of multiple modified search structures. Subsequent molecular replacement using the computer program *EPMR* (Kissinger *et al.*, 1999) with the atomic coordinates of the *A* molecule of the *D. pteronyssinus* mite allergen as the search molecule (Derewenda *et al.*, 2002; PDB code 1ktj), generated a robust single solution in space group $I4_122$. Optimization of the rotation and translation functions yielded an *R* factor of 0.434 and a correlation coefficient of 0.599 in the 15–4.0 Å resolution shell. Subsequent annealing refinement using the computer package *CNS* (Brünger *et al.*, 1998) appeared promising, with an overall *R* factor for test and working data sets of 0.364 and 0.332, respectively (for all data in the resolution range 30.0–2.4 Å). The unit-cell packing with one molecule of Der f 2 in the asymmetric unit is reasonable and does not permit the positioning of a second search molecule.

Although the crystals of Der f 2 diffract to reasonable resolution (2.4 Å or better), they are radiation-sensitive even when frozen. The strong diffuse scattering noted in the

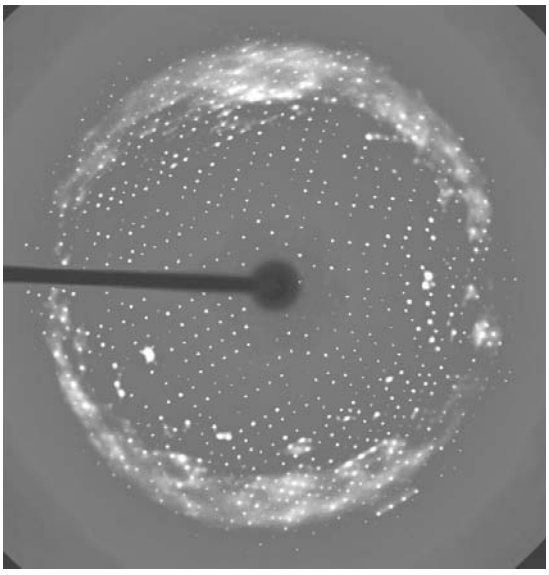

**Figure 2**
Imaging-plate frame collected from a cryocooled Der f 2 crystal demonstrating high mosiacity/diffuse scattering. The crystals diffract to a nominal resolution of 2.4 Å.

diffraction images suggests that either large segments or entire regions of the protein are mobile (Fig. 2). Based on the presence of one protein molecule in the asymmetric unit and a molecular weight of 15.8 kDa, the volume per unit mass is 3.65 $Å^3$ $Da^{-1}$. This value marginally exceeds the range (1.7–3.5 $Å^3$ $Da^{-1}$) typically found for crystallized proteins. The calculated solvent content of 66% is consistent with a molecule that is only loosely restrained by packing contacts (Matthews, 1968).

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