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Crystallization and preliminary crystallographic data of a Fel d 1 (1+2) construct corresponding to the major allergen from cat

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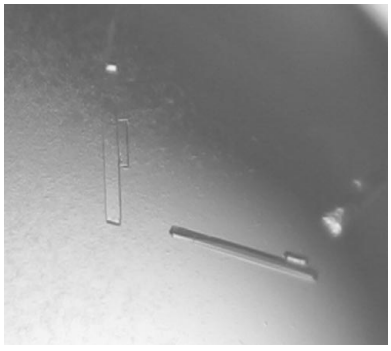

The domestic cat (*Felis domesticus*) is one of the most important causes of allergic disease worldwide. A homologue of the major allergen Fel d 1 was created by linking the two chains that compose the protein. Fel d 1 (1+2) was expressed in *Escherichia coli* and subsequently purified using three chromatographic steps. Crystals of Fel d 1 (1+2) were obtained using the hanging-drop vapour-diffusion method in 22.5% PEG 3350, 0.5 *M* $CaCl_2$. The crystals belong to space group *P*1, with unit-cell parameters $a = 38.5$, $b = 42.9$, $c = 49.0$ Å, $\alpha = 70.7$, $\beta = 80.5$, $\gamma = 81.5^\circ$, and diffract to 1.6 Å resolution.

1. Introduction

Sensitization to the domestic cat (*Felis domesticus*) is a common cause of allergic disease, with a prevalence of approximately 10% in the westernized world (Freidhoff *et al.*, 1984; Roost *et al.*, 1999). The severity of symptoms ranges from mild rhinitis to life-threatening asthmatic responses. Cat allergens are abundant in society even in environments where cats are not present, such as schools and public transportation (Partti-Pellinen *et al.*, 2000; Almqvist *et al.*, 2001), and are therefore difficult to avoid. A careful structural and functional characterization of the major allergen is necessary to improve diagnostics and therapy of cat allergy.

The most prominent and potent allergen in cat dander, termed Fel d 1 (*Felis domesticus* allergen 1), was identified more than three decades ago (Ohman *et al.*, 1974). Fel d 1 elicits IgE responses in 95% of patients with cat allergy (van Ree *et al.*, 1999). The allergen is present in cat saliva and pelt, as well as in perianal and lachrymal glands (Anderson *et al.*, 1985; Bartholome *et al.*, 1985; van Milligen *et al.*, 1990). Fel d 1 is a 35 kDa tetrameric glycoprotein (Kristensen *et al.*, 1997) formed by two heterodimers (Duffort *et al.*, 1991). Each dimer is composed of two chains derived from independent genes, chain 1 (70 residues) and chain 2 (90 or 92 residues) (Morgenstern *et al.*, 1991; Griffith *et al.*, 1992). The two isoforms of the second chain are expressed in the skin and the saliva, respectively (Griffith *et al.*, 1992). The chains are linked by three disulfide bonds, formed between Cys3 of chain 1 and Cys73 of chain 2, between Cys44 of chain 1 and Cys48 of chain 2, and between Cys70 of chain 1 and Cys7 of chain 2 (Bond *et al.*, 1993; Kristensen *et al.*, 1997).

Using a direct linkage of chain 1 to chain 2 [construct Fel d 1 (1+2)] and chain 2 to chain 1 [construct Fel d 1 (2+1)], we have been able to establish *in vitro* conditions for the appropriate refolding of recombinant Fel d 1 fused homologues (Fig. 1). The refolded recombinant Fel d 1 proteins display an identical disulfide-bonding pattern and a comparable secondary structure to the native protein as revealed by

Figure 1
Expression constructs used for Fel d 1 production. (*a*) Fel d 1 (1+2). (*b*) Fel d 1 (2+1). Chains 1, 2 and the six-histidine tags are coloured white, grey and black, respectively.

circular dichroism (Grönlund *et al.*, 2003). Both proteins form dimers of the fused chains, corresponding to the native tetramer. Most importantly, the *in vitro* immunoreactivity of both stable recombinant Fel d 1 proteins is indistinguishable from the native allergen (Grönlund *et al.*, 2003). We have recently succeeded in solving the structure of the Fel d 1 (2+1) heterodimer to a resolution of 1.9 Å (Kaiser, Grönlund, Sandalova, Ljunggren, Schneider *et al.*, 2003; Kaiser, Grönlund, Sandalova, Ljunggren, van Hage-Hamsten *et al.*, 2003). However, while both native Fel d 1 and Fel d 1 (2+1) are purified as a tetramer/dimer, no dimer was formed in the crystallization conditions (16% MPD, 0.1 *M* sodium acetate pH 4.8) obtained for Fel d 1 (2+1). In addition, no electron density could be assessed for the Fel d 1 (2+1) region corresponding to the C-terminus of chain 2, indicating disorder in that part of the molecule. This disorder could also be attributed to the artificial connection of chain 2 to chain 1.

The purpose of the present study was to establish a system for structural studies of the dimeric form of the major cat allergen Fel d 1 as well as to attempt to determine its complete structure. We report here the conditions for successful refolding, purification and crystallization of Fel d 1 (1+2). The structure will provide deepened insights into the function of Fel d 1 and facilitate the improvement of candidates for cat-allergy immunotherapy.

2. Materials and methods

2.1. Protein cloning, expression and purification of Fel d 1 (1+2)

The Fel d 1 (1+2) construct was created by directly linking the C-terminal residue of chain 1 (Cys70) with the N-terminal residue of chain 2 (Val70) using overlapping oligonucleotides in PCR (Fig. 1). The sequenced PCR product was cloned into the pET 20b expression vector (Studier *et al.*, 1990) and transformed into BL21 (DE3) pLysS (Novagen). Fel d 1 (1+2) was expressed following induction with IPTG as a six-histidine fusion protein in *Escherichia coli* strain BL21 (DE3) pLysS and was purified from inclusion bodies. The protein was solubilized in 20 m*M* Tris–HCl containing 6 *M* guanidine–HCl, 0.5 *M* NaCl and 5 m*M* imidazole pH 8.0 and bound to a Hi-Trap Ni^{2+}-chelate affinity column (Amersham Biosciences). The column buffer was changed to 6 *M* urea, 20 m*M* Tris–HCl, 0.5 *M* NaCl, 20 m*M* imidazole pH 8.0 and a linear 12-column gradient to 20 m*M* Tris–HCl, 0.5 *M* NaCl, 20 m*M* imidazole pH 8 was used. The protein was eluted with 20 m*M* Tris–HCl, 0.5 *M* NaCl, 0.5 *M* imidazole pH 8.0. Fel d 1 (1+2) was further purified to homogeneity by size-exclusion chromatography followed by MonoQ ion-exchange chromatography (Amersham Biosciences). The concentration of the final product was determined spectrophotometrically and the purity of the product was assessed by SDS–PAGE under denaturing conditions (Laemmli, 1970) and gel filtration.

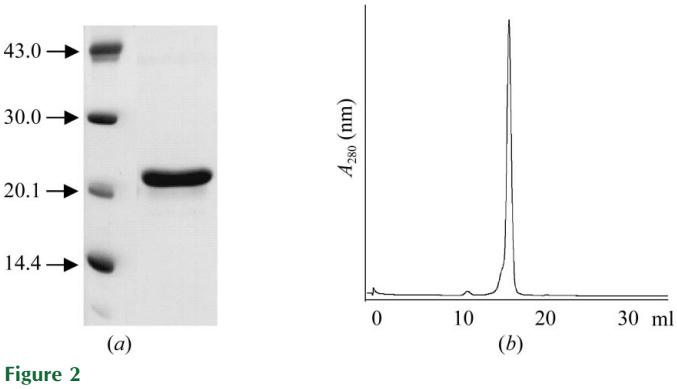

Figure 2
(*a*) Proteins separated by SDS–PAGE (15% gel) under denaturing and reducing conditions, stained with Coomassie brilliant blue. Lane 1, molecular-weight markers in kDa; lane 2, purified Fel d 1 (1+2). (*b*) Fel d 1 (1+2) elution profile from gel-filtration FPLC Superdex 200 column (Amersham Biosciences) in 0.5 ml min^{-1} PBS. The elution was monitored at 280 nm.

2.2. Crystallization of Fel d 1 (1+2)

Crystals were obtained by hanging-drop vapour diffusion. Crystal Screens (Hampton Research) were used to establish initial crystallization conditions for Fel d 1 (1+2), which were then refined in a finer grid. Typically, 2 µl 5 mg ml^{-1} protein in 20 m*M* Tris–HCl pH 7.5 was mixed in a 1:1 ratio with the crystallization reservoir solution (22.5% PEG 3350, 0.5 *M* $CaCl_2$) and allowed to equilibrate both at room temperature and at 277 K.

2.3. Data collection and processing

A data set for Fel d 1 (1+2) was collected at beamline I711 at MAX-II, Lund. A total of 253 images were collected with 1.5° oscillation per image. The space group and unit-cell parameters were determined using the autoindexing option of *MOSFLM* (Leslie, 1999) and scaling and reduction of the data were performed using programs from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994). The space group and unit-cell parameters were determined using the autoindexing option of *MOSFLM*.

3. Results and discussion

The Fel d 1 (1+2) construct is composed of 170 amino acids with a calculated molecular weight of 19 177 Da and a theoretical pI of 5.0. The protein was produced in *E. coli*, refolded *in vitro* and isolated using an Ni^{2+}-chelate column, gel-filtration and ion-exchange chromatography. Recombinant Fel d 1 (1+2) was purified as a dimer (corresponding to the native tetramer) and a minimum of 5 mg of highly purified refolded protein was obtained from 1 l bacterial culture (Fig. 2). As for Fel d 1 (2+1), the stable recombinant Fel d 1 (1+2) protein displays an identical disulfide-bonding pattern to the native protein and a comparable secondary structure as revealed by circular dichroism (Grönlund *et al.*, 2003). Most importantly, it also displays a similar immunoreactivity *in vitro* to the native allergen (Grönlund *et al.*, 2003). The initial Fel d 1 (1+2) crystals appeared after four weeks in 20% PEG 3350, 0.1 *M* $CaCl_2$ at 295 K. The size and quality of the crystals were improved by increasing the salt and precipitant concentration and the final crystals were obtained in 22.5% PEG 3350 and 0.5 *M* $CaCl_2$ at 295 K (Fig. 3).

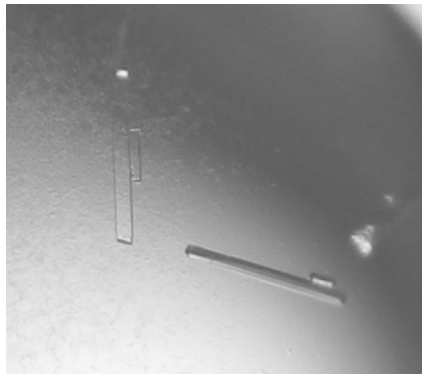

Figure 3
Photograph of typical crystals of Fel d 1 (1+2) in 22.5% PEG 3350 and 0.5 *M* $CaCl_2$. The longest dimension is ∼0.2 mm.

Table 1
Data-collection statistics.

Values in parentheses are for the highest resolution shell.

X-ray source	I711
Wavelength (Å)	1.087
Resolution (Å)	37.8–1.6
Space group	*P*1
Unit-cell parameters	
a (Å)	38.5
b (Å)	42.9
c (Å)	49.0
α (°)	70.7
β (°)	80.5
γ (°)	81.5
Total No. unique reflections	33386
No. observed reflections	135810
Completeness (%)	92.1 (87.5)
R_{sym}	0.06 (0.26)
$I/\sigma(I)$	17.4 (4.2)
Mosaicity (°)	0.6

The native crystals diffracted to 1.6 Å resolution using synchrotron radiation. The statistics of the data set are listed in Table 1. The crystals belong to space group *P*1, with unit-cell parameters $a = 38.5$, $b = 42.9$, $c = 49.0$ Å, $\alpha = 70.7$, $\beta = 80.5$, $\gamma = 81.5^\circ$. The average *B* factor derived from a Wilson plot was 21 Å^2. The crystals are very likely to contain two Fel d 1 molecules per asymmetric unit, which would give a Matthews coefficient of $V_M = 2.0$ Å^3 Da^{-1}, corresponding to 37% solvent content.

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References

Almqvist, C., Wickman, M., Perfetti, L., Berglind, N., Renstrom, A., Hedren, M., Larsson, K., Hedlin, G. & Malmberg, P. (2001). *Am. J. Respir. Crit. Care Med.* **163**, 694–698.
Anderson, M. C., Baer, H. & Ohman, J. L. Jr (1985). *J. Allergy Clin. Immunol.* **76**, 563–569.
Bartholome, K., Kissler, W., Baer, H., Kopietz-Schulte, E. & Wahn, U. (1985). *J. Allergy Clin. Immunol.* **76**, 503–506.
Bond, J. F., Brauer, A. W., Segal, D. B., Nault, A. K., Rogers, B. L. & Kuo, M. C. (1993). *Mol. Immunol.* **30**, 1529–1541.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Duffort, O. A., Carreira, J., Nitti, G., Polo, F. & Lombardero, M. (1991). *Mol. Immunol.* **28**, 301–309.
Freidhoff, L. R., Meyers, D. A. & Marsh, D. G. (1984). *J. Allergy Clin. Immunol.* **73**, 490–499.
Griffith, I. J., Craig, S., Pollock, J., Yu, X. B., Morgenstern, J. P. & Rogers, B. L. (1992). *Gene*, **113**, 263–268.
Grönlund, H., Bergman, T., Sandström, K., Alvelius, G., Reininger, R., Verdino, P., Hauswirth, A., Liderot, K., Valent, P., Spitzauer, S., Keller, W., Valenta, R. & van Hage-Hamsten, M. (2003). *J. Biol. Chem.* **278**, 40144–40151.
Kaiser, L., Grönlund, H., Sandalova, T., Ljunggren, H. G., Schneider, G., van Hage-Hamsten, M. & Achour, A. (2003). *Acta Cryst.* D**59**, 1103–1105.
Kaiser, L., Grönlund, H., Sandalova, T., Ljunggren, H. G., van Hage-Hamsten, M., Achour, A. & Schneider, G. (2003). *J. Biol. Chem.* **278**, 37730–37735.
Kristensen, A. K., Schou, C. & Roepstorff, P. (1997). *Biol. Chem.* **378**, 899–908.
Laemmli, U. K. (1970). *Nature (London)*, **227**, 680–685.
Leslie, A. G. W. (1999). *Acta Cryst.* D**55**, 1696–1702.
Milligen, F. J. van, Vroom, T. M. & Aalberse, R. C. (1990). *Int. Arch. Allergy Appl. Immunol.* **92**, 375–378.
Morgenstern, J. P., Griffith, I. J., Brauer, A. W., Rogers, B. L., Bond, J. F., Chapman, M. D. & Kuo, M. C. (1991). *Proc. Natl Acad. Sci. USA*, **88**, 9690–9694.
Ohman, J. L., Lowell, F. C. & Bloch, K. J. (1974). *J. Immunol.* **113**, 1668–1677.
Partti-Pellinen, K., Marttila, O., Makinen-Kiljunen, S. & Haahtela, T. (2000). *Allergy*, **55**, 65–68.
Ree, R. van, van Leeuwen, W. A., Bulder, I., Bond, J. & Aalberse, R. C. (1999). *J. Allergy Clin. Immunol.* **104**, 1223–1230.
Roost, H. P., Künzli, N., Schindler, C., Jarvis, D., Chinn, S., Perruchoud, A. P., Ackermann-Liebrich, U., Burney, P. & Wüthrich, B. (1999). *J. Allergy Clin. Immunol.* **104**, 941–947.
Studier, F. W., Rosenberg, A. H., Dunn, J. J. & Dubendorff, J. W. (1990). *Methods Enzymol.* **185**, 60–89.