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Crystallization and preliminary crystallographic analysis of the extracellular fragment of FcαRI/CD89

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Crystals of the extracellular fragment of FcαRI/CD89 have been grown at 291 K using PEG 8000 as precipitant. The diffraction pattern of the selenomethionine (SeMet) derivative crystal extended to 2.1 Å resolution at SPring-8, Japan. The crystals belong to space group $C222_1$, with unit-cell parameters a = 59.0, b = 69.4, c = 106.3 Å, $\alpha = \beta = \gamma = 90^\circ$. The presence of one molecule per asymmetric unit gives a crystal volume per protein mass (V_M) of 3.12 Å^3 Da^{-1} and a solvent content of 60.2% by volume. A full set of X-ray diffraction data were collected to 2.1 Å from an SeMet-derivative crystal.

1. Introduction

Immunoglobulin A is the most prominent antibody class at mucosal surfaces and also the second most prevalent circulating antibody class in human serum (van Egmond *et al.*, 2001). Receptors for IgA play a significant role *in vivo* in maintaining the integrity of immune responses in systemic and mucosal compartments. This receptor appears to play an important role in immunity by linking the IgA response to powerful cellular effector mechanisms (Monteiro & van de Winkel, 2003). The binding of serum IgA to its Fc receptor (FcαRI or CD89) can trigger a variety of processes, including endocytosis, phagocytosis, superoxide generation, antibody-dependent cell-mediated cytotoxicity, IgA-IC degradation and release of enzymes/cytokines and inflammatory mediators (Morton *et al.*, 1996). FcαRI is a member of the multichain immune-recognition receptor (MIRR) family. It expression is restricted to the myeloid lineage cells, which including neutrophils, eosinophils, most monocytes/macrophages, interstitial dendritic cells, Kupffer cells and cell lines corresponding to these cell types (Morton *et al.*, 1996; van Egmond *et al.*, 2000; Monteiro & van de Winkel, 2003). It is also expressed in interstitial-type dendritic cells (Geissmann *et al.*, 2001) and there are several reports of its possible expression on mesangial cells in the kidney (Barratt *et al.*, 2000; Suzuki *et al.*, 1999; Leung *et al.*, 2000; Westerhuis *et al.*, 1999; Patry *et al.*, 1996). FcαRI comprises two extracellular immunoglobulin-like domains, a transmembrane region and a short cytoplasmic tail (Maliszewski *et al.*, 1990). Despite the lack of any known signalling component of FcαRI, it still plays an active role in triggering Ca^{2+} mobilization and neutrophil degranulation by associating with the promiscuous FcγR chain (van Egmond *et al.*, 2001; Morton *et al.*, 1996). Recent studies have indicated that the short intracellular domain of FcαRI is important for its regulation by cytokines (Bracke *et al.*, 2001). The immunoglobulin-binding affinity of FcαRI was reported to be $\sim 10^{-6}\,M^{-1}$, making FcαRI a low-affinity receptor. Accordingly, FcαRI binds complexed IgA, whereas monomeric IgA presumably interacts only transiently (Morton *et al.*, 1996; Wines *et al.*, 1999). Unusually for FcRs, the ligand-binding site for IgA is presumably located in the first extracellular domain of FcαRI (Wines *et al.*, 2001), whereas other leukocyte FcR ligand-binding sites are in the second extracellular Ig-like domain (Sondermann *et al.*, 2000; Garman *et al.*, 2000; Radaev *et al.*, 2001). The FcαRI-docking site on IgA is located at or close to the $C_\alpha 2/C_\alpha 3$ boundary (Pleass *et al.*, 2001).

The recent crystal structures of FcεRα (Garman *et al.*, 1998), FcγRIIa (Maxwell *et al.*, 1999), FcγRIIb (Sondermann *et al.*, 1999) and FcγRIII (Zhang *et al.*, 2000) have each revealed a conserved Ig-like structure with a small hinge angle between the two Ig-like domains, which is unique to the Fc receptors. In the present study, the crystallization and preliminary crystallographic analysis of the extracellular fragment of FcαRI are reported. The structure of this protein may be helpful in the illustration of the function of the FcαRI protein.

2. Materials and methods

2.1. Protein expression and purification

The extracellular ligand-binding domain residues 1–207 of human FcαRI were subcloned into a Novagen pET-28a vector using the *Nco*I and *Xho*I restriction sites and *Escherichia coli* strain BL21 (DE3). Two additional amino acids (Met-Ala) were added to the 5′ end of the gene and a histidine tag (His_6) was added to the 3′ end to facilitate expression and purification. The protein was

first expressed in an inclusion-body form and then reconstituted *in vitro*. In brief, cells containing the FcαRI-expressing plasmid were grown in Terrific Broth media and induced with 1.0 m*M* IPTG at an approximate OD_{600} of 0.8 for 6.0 h. Once harvested, the isolation of the inclusion bodies was started with an intense combined lysozyme/sonication procedure to open virtually all cells. The inclusion bodies were washed with 2% Triton X-100 in washing buffer (25 m*M* Tris–HCl, 500 m*M* NaCl, 1 m*M* DTT pH 8.0) with a short sonication; the inclusion bodies were then washed with 2 *M* NaCl in washing buffer with a further short sonication, yielding a product with a purity of >85% as estimated by SDS–PAGE. The inclusion bodies were dissolved in a buffer containing 6 *M* guanidine hydrochloride and 10 m*M* DTT and incubated for 2 h to unfold the misfolded inclusion-body protein completely at room temperature. Refolding was achieved by diluting the guanidine-dissolved inclusion bodies dropwise with stirring into refolding buffer (0.1 *M* Tris–HCl, 1.6 *M* guanidine hydrochloride, 5 m*M* reduced glutathione, 0.5 m*M* oxidized glutathione pH 8.5) at 277 K. The mixture was stirred for 2–3 d until the concentration of free thiol groups was reduced to 1 m*M*. The renatured FcαRI was applied to a Q-Sepharose High-Performance ion-exchange column (Pharmacia) (buffer *A*, 25 m*M* Tris–HCl pH 8.0, 50 m*M* NaCl, 1 m*M* β-mercaptoethanol; buffer *B*, 25 m*M* Tris–HCl pH 8.0, 1.0 *M* NaCl, 1 m*M* β-mercaptoethanol). The protein was concentrated to about 20 mg ml^{-1} using an Ultrafree 5000 MWCO unit filter (Millipore) and applied to a Superdex-75 column (Pharmacia) equilibrated in crystallization buffer (100 m*M* NaCl, 100 m*M* Tris–HCl pH 8.0, 100 m*M* imidazole, 1 m*M* DTT, 1 m*M* NaN_3). The purified protein was analyzed on SDS–PAGE and native PAGE, which showed that the protein was >95% pure. Dynamic light-scattering data showed the protein had 70–80% homogeneity as a monomer.

The expression, refolding and purification of the selenomethionine-labelled protein were similar to those used for the native protein.

2.2. Crystallization

The purified protein was concentrated to 12 mg ml^{-1} using an Ultrafree 5000 MWCO unit filter. Protein concentrations were estimated spectroscopically from the absorbance at 280 nm, assuming an A_{280} of 1.30 for a 1.0 mg ml^{-1} solution. Initial screening was performed at 291 K by the hanging-drop vapour-diffusion method using sparse-matrix (Jancarik & Kim, 1991) screening kits from Hampton Research (Crystal Screen kits I and II), followed by refinement of the conditions through the variation of precipitant, pH, protein concentration and additives. Typically, 3 µl droplets were prepared on siliconized cover slips by mixing 1.5 µl protein solution and 1.5 µl reservoir solution. The purified selenomethionine-derivative protein was concentrated to 9 mg ml^{-1}. Crystallization trials were set up based on the optimum conditions used for native protein.

2.3. X-ray crystallographic studies

Preliminary diffraction data sets were collected in-house on a Rigaku RU-2000 rotating-anode Cu $K\alpha$ X-ray generator at 48 kV and 98 mA (λ = 1.5418 Å) with a MAR 345 mm image-plate detector. The beam was focused using Osmic mirrors. For a more detailed analysis, flash-cooled crystals were used. Crystals were immersed in a cryoprotectant solution consisting of mother liquor supplemented with 15% ethylene glycol, picked up in a loop and flash-cooled in a stream of nitrogen gas cooled to 100 K. All intensity data were indexed, integrated and scaled using the *HKL* programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). A single selenomethionine (SeMet) derivative crystal was soaked in cryoprotectant solution and flash-frozen in liquid N_2. Diffraction data were collected on beamline BL41XU at SPring-8. The exposure time was 8 s, the crystal-to-detector distance was 150 mm and the oscillation range per frame was 1°.

3. Results

The final purified protein was confirmed to be homogenous by SDS–PAGE, native PAGE and dynamic light-scattering analysis and was fit for use in crystallization trials. Small cluster crystals appeared after two weeks from several different conditions from Crystal Screen kits I and II (Hampton Research) containing PEG 4000 and PEG 8000 as precipitants (condition No. 37 of kit I and No. 37 of kit II, respectively). The conditions were further optimized by variation of the precipitants, buffer pH and protein concentration.

We found the crystals grew more quickly and to an optimum size using 12% PEG 8000 in 100 m*M* Na HEPES buffer pH 7.6 containing 8%(*v*/*v*) ethylene glycol as an additive with a protein concentration of 15 mg ml^{-1} and vapour-equilibrating against 150 µl reservoir solution. However, the crystals were badly twinned (Fig. 1*a*) and were unsuitable for data collection. The crystals were refined by adjusting the concentration, pH value, precipitant concentration and additives. The crystallographic PCR (Prompt Crystallization Reaction, a systematic micro/macroseeding method) system was used to refine the crystals (Yang & Rao, 2003). Large 'aircraft-

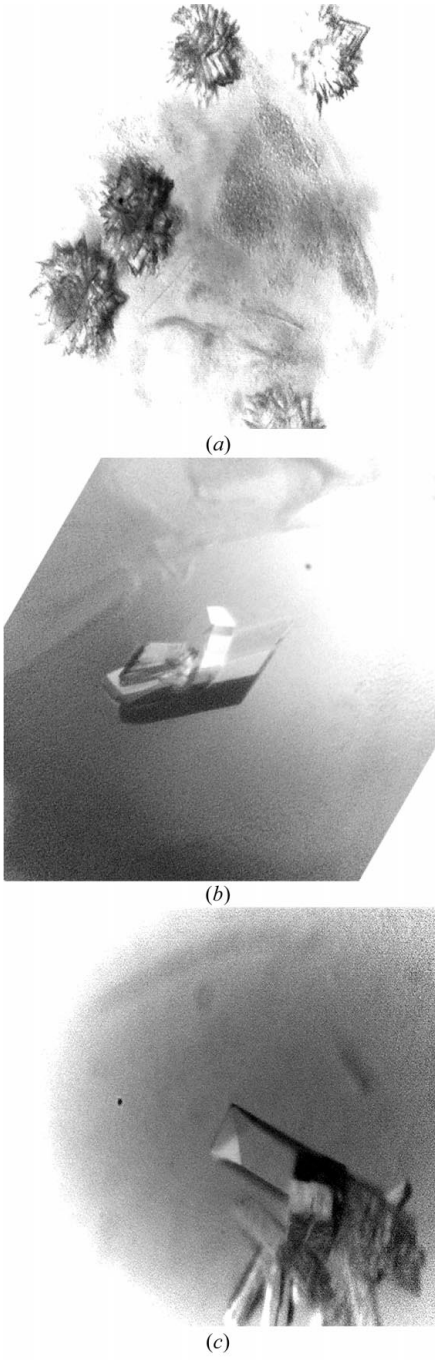

Figure 1
Crystals of CD89. (*a*) Twinned crystals. (*b*) Large 'aircraft-carrier' shaped crystal. (*c*) SeMet-derivative crystal. This crystal is 0.3 × 0.4 × 0.8 mm in size.

Table 1
Data-collection and processing statistics.

Values in parentheses are for the highest resolution shell.

	λ_1 (peak)	λ_2 (edge)	λ_3 (remote)
Wavelength (Å)	0.9798	0.9800	0.9000
Resolution range (Å)	50–2.1	50–2.1	50–2.1
Completeness (%)	100 (100)	99.9 (99.5)	100 (99.9)
Total reflections	95646	95900	95489
Unique reflections	13117	13133	13108
R_{sym}† (%)	6.8 (20.7)	5.2 (18.9)	6.1 (23.4)
$I/\sigma(I)$	15.9 (7.4)	16.2 (7.7)	15.2 (6.7)
Redundancy	7.3 (7.1)	7.3 (7.1)	7.3 (7.0)
Crystal-to-detector distance (mm)	150	150	160
f'/f'' (e)	−7/7	−12/2	−1.6/3.3

† $R_{sym} = 100\sum|I_i - \langle I\rangle|/\sum_i$, where I_i is the intensity of the ith observation

carrier' shaped crystals (Fig. 1*b*) were obtained which were reproducible and suitable for X-ray diffraction. The crystals grew to dimensions of 0.4 × 0.5 × 0.8 mm from reservoir solution comprising 11.4% PEG 8000 in 100 m*M* Na HEPES buffer pH 7.64 containing 8.0%(*v*/*v*) ethylene glycol, 3.0%(*v*/*v*) DMSO and 50 m*M* $MgCl_2$ as an additive with a protein concentration of 11 mg ml^{-1}. The crystals grown from the optimum reservoir solution condition are compact and stable, as demonstrated by their diffraction to 1.3 Å at ESRF following storage for about 20 d at 291 K. The crystals belong to space group $C222_1$, with unit-cell parameters a = 59.0, b = 69.3, c = 106.1 Å, $\alpha = \beta = \gamma = 90.0^\circ$. There is one molecule in the asymmetric unit. R_{merge} is 6.3% and the completeness is 98.1%.

Since there are four methionine residues in the FcαRI protein, the MAD method was considered. SeMet-derivative crystals (Fig. 1*c*) were obtained using similar conditions to those for the native protein, except for a lower pH (0.1 *M* HEPES pH 7.45) and protein concentration (9 mg ml^{-1}). MAD data were collected from a single SeMet-derivative crystal to 2.1 Å resolution (λ = 0.9798, 0.9800, 0.9000 Å). The statistics of data collection from SeMet-derivative crystals are shown in Table 1. The presence of one molecule per asymmetric unit gives a Matthews coefficient (V_M; Matthews, 1968) of 3.12 Å^3 Da^{-1} and a solvent content of 60.2% by volume.

Structure determination of the FcαRI protein is currently under way.

Note added in proof: After submission of this manuscript, it came to the authors' attention that another structural report on FcαRI was in press (Herr *et al.*, 2003).

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