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Crystallization and preliminary X-ray diffraction analysis of human IL-22 bound to its soluble decoy receptor IL-22BP

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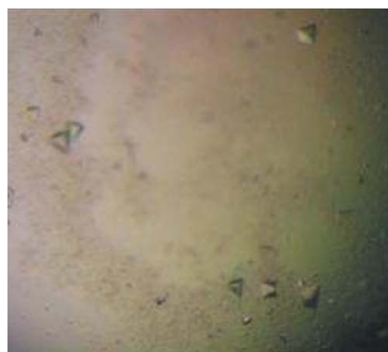
Interleukin-22 (IL-22) is a pleiotropic cytokine that is involved in inflammatory responses. Human IL-22 was incubated with its soluble decoy receptor IL-22BP (IL-22 binding protein) and the IL-22–IL-22BP complex was crystallized in hanging drops using the vapour-diffusion method. Suitable crystals were obtained from polyethylene glycol solutions and diffraction data were collected to 2.75 Å resolution. The crystal belonged to the tetragonal space group $P4_1$, with unit-cell parameters $a = b = 67.9$, $c = 172.5$ Å, and contained two IL-22–IL-22BP complexes per asymmetric unit.



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1. Introduction

Interleukin-22 (IL-22) is a pleiotropic cytokine that is mainly produced by activated T cells, including the recently identified T_H17 lineage of $CD4^+$ effector lymphocytes (Dumoutier *et al.*, 2000; Xie *et al.*, 2000; Liang *et al.*, 2006). IL-22 is involved in the generation of inflammatory and immune responses that lead to the development of several illnesses such as psoriasis (Boniface *et al.*, 2007), rheumatoid arthritis (Ikeuchi *et al.*, 2005) and Crohn's disease (Schmechel *et al.*, 2008; Wolk *et al.*, 2004), amongst others. Despite its pro-inflammatory effects in T-cell-mediated diseases, IL-22 plays an anti-inflammatory hepatoprotective role in T-cell-mediated liver injury (Radaeva *et al.*, 2004). Moreover, IL-22 has also been implicated in the physiological defence against pathogens by stimulating the secretion of antibacterial proteins and promoting epithelial repair (Wolk *et al.*, 2004; Aujla *et al.*, 2008; Zheng *et al.*, 2008).

Human IL-22 is a 20 kDa glycoprotein that folds into a compact bundle of six antiparallel α-helices (Nagem *et al.*, 2002; Xu *et al.*, 2005). IL-22 signals through its binding to the extracellular domains of the IL-22R1 (CRF2-9) and IL-10R2 (CRF2-4) receptor chains (Xie *et al.*, 2000; Kotenko *et al.*, 2001*a*). These receptor chains are structurally related to the class II cytokine receptor chain IL-10R1; the IL-10R2 chain is also used by other cytokines such as IL-10, IL-26, IL-28 and IL-29 (Donnelly *et al.*, 2004; Renauld, 2003). The specificity of IL-22 binding depends on IL-22R1 and the sequential binding of IL-10R2 to IL-22–IL-22R1 stabilizes IL-22 within the receptor complex and leads to effective signal transduction (Kotenko *et al.*, 2001*a*; Li *et al.*, 2004). The three-dimensional structure of the IL-22–IL-22R1 complex has recently been published and revealed that IL-22 has distinct binding sites for the extracellular domains of IL-22R1 and IL-10R2 (Jones *et al.*, 2008; Bleicher *et al.*, 2008).

IL-22 also binds to a soluble receptor named IL-22BP (IL-22 binding protein), which shares 34% amino-acid sequence identity with the extracellular domain of the IL-22R1 chain (Dumoutier *et al.*, 2001; Kotenko *et al.*, 2001*b*; Xu *et al.*, 2001). IL-22BP is a secreted protein that lacks both transmembrane and intracellular domains; the mature protein contains 210 amino-acid residues, four cysteine residues, five potential *N*-glycosylation sites and two tandem FBN-III domains (Dumoutier *et al.*, 2001; Weiss *et al.*, 2004). It has been shown that IL-22BP binds to IL-22 with high affinity and thus functions as a natural antagonist of IL-22 signalling by preventing the assembly of the IL-22 functional complex (Dumoutier *et al.*, 2001; Kotenko *et al.*,

2001*b*; Xu *et al.*, 2001). Moreover, it has been reported that the binding of IL-22 to IL-22BP might prevent inflammation that could provoke rejection of the embryo in pregnancy (Gruenberg *et al.*, 2001), indicating a potential role of IL-22BP in the regulation of IL-22 inflammatory responses. In this paper, the crystallization and preliminary X-ray diffraction results of the IL-22–IL-22BP complex are described.

2. Materials and methods

2.1. Protein expression and purification

Recombinant human IL-22 (Gene ID 50616; residues 29–179) was expressed in *Escherichia coli* and purified as reported previously (Nagem *et al.*, 2002). Recombinant human IL-22BP (Gene ID 116379) was produced in *E. coli* as follows. The IL-22BP sequence (corresponding to amino acids Gly21–Pro231) was amplified by PCR from a cDNA clone and cloned into the pET9a plasmid (Stratagene, La Jolla, California, USA). *E. coli* strain BL21-Codon Plus-(DE3)-RIL (Stratagene) was used as the expression host. The recombinant protein was obtained as inclusion bodies. The inclusion bodies were solubilized in 6 *M* guanidine chloride and reduced with 50 m*M* DTT in Tris buffer pH 8.5. The IL-22BP protein was refolded by direct dilution of the solubilized inclusion bodies in the following mixture: 50 µg ml^{-1} IL-22BP, 50 m*M* Bicine pH 8.5, 0.2 *M* L-arginine, 0.5 *M* $MgSO_4.7H_2O$, 1 m*M* reduced glutathione and 0.1 m*M* oxidized glutathione. The solution was incubated for one week at 277 K. 10%(*v*/*v*) glycerol; 1 m*M* CHAPS was added and the mixture was concentrated by ultrafiltration in an Amicon chamber with a YM10 membrane before purification on a Superdex200 (Amersham Pharmacia Biotech) gel-filtration column. The protein was eluted with 25 m*M* Bicine pH 9.5, 150 m*M* NaCl, 1 m*M* CHAPS and 10%(*v*/*v*) glycerol.

The IL-22–IL-22BP binary complex was formed by incubating IL-22 with IL-22BP in a 1.2:1 molar ratio at 277 K overnight. The complex was purified by gel-filtration chromatography on a Superdex200 column buffer consisting of 25 m*M* Bicine pH 9.5, 150 m*M* NaCl and 1 m*M* CHAPS. The purified complex was concentrated to 5 mg ml^{-1} in the same elution buffer for crystallization trials.

2.2. Crystallization and data collection

Crystallization experiments employed the hanging-drop vapour-diffusion method and were performed at 291 K. Preliminary screening of the crystallization conditions was performed using a sparse-matrix screen (Crystal Screens I and II from Hampton Research; MPD Suite, PEGs Suite and Classics Suite from Qiagen). Briefly, 1 µl IL-22–IL-22BP complex (5 mg ml^{-1} in 25 m*M* Bicine pH 9.5, 150 m*M* NaCl and 1 m*M* CHAPS) and 1 µl reservoir solution were mixed and the 2 µl drops were equilibrated against 600 µl reservoir solution. Small crystals were found in condition No. 30 from Crystal Screen II and condition No. 93 from Classics Suite. In order to improve the crystal size, the pH and precipitant concentration were varied. Prior to data collection, a single IL-22–IL-22BP crystal was soaked in a cryoprotectant solution composed of crystallization solution containing 10%(*v*/*v*) glycerol and then flash-cooled in a nitrogen stream (100 K) on the W01B-MX2 beamline at LNLS (Campinas, São Paulo, Brazil). The beam energy was set to 9.5 keV. A data set of 65 images was collected using a 1° oscillation step to a maximum resolution beyond 2.75 Å using a MARmosaic225 detector. The data set was indexed, reduced, merged and scaled using *MOSFLM* (Leslie, 1992) and *SCALA* (Collaborative Computational Project, Number 4, 1994).

3. Results and discussion

The IL-22–IL-22BP complex crystallized 7 d after initial setup using the hanging-drop vapour-diffusion method at 291 K. Small crystals were obtained from reservoir solution containing 10%(*w*/*v*) PEG 6000, 5%(*v*/*v*) MPD and 0.1 *M* HEPES pH 6.8 (Fig. 1). Although the IL-22–IL-22BP crystals were rather small (with a typical dimension of 25 µm), they were suitable for X-ray diffraction experiments and a complete 2.75 Å resolution data set was collected (Fig. 2, Table 1). The data-set processing statistics revealed that the IL-22–IL-22BP crystal belonged to a primitive tetragonal lattice, with unit-cell parameters $a = b = 67.9$, $c = 172.5$ Å and systematic absences along the *l* axis consistent with space group $P4_1$ or $P4_3$. Initial analysis of the crystal solvent content using the Matthews coefficient (Matthews,

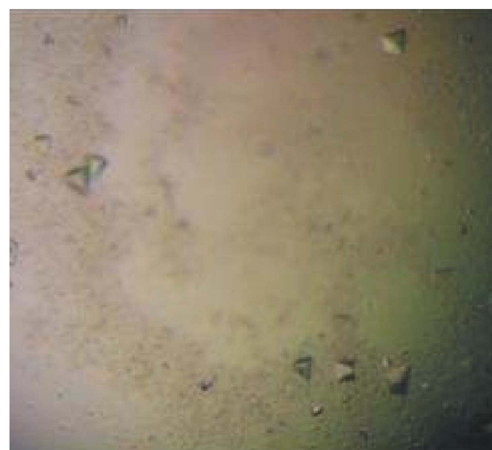

Figure 1
Crystals of the IL-22–IL-22BP complex. Typical crystal dimensions were 25 µm.

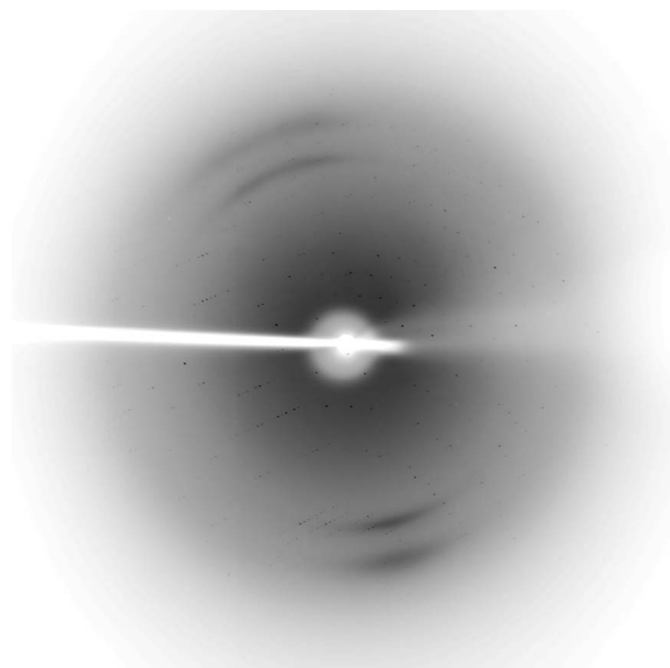

Figure 2
Typical diffraction image of an IL-22–IL-22BP crystal.

Table 1
X-ray data-collection statistics.

Values in parentheses are for the highest resolution shell.

Space group	$P4_1$
Unit-cell parameters (Å)	$a = b = 67.9$, $c = 172.5$
Wavelength (Å)	1.43
Resolution range (Å)	22.6–2.75 (2.9–2.75)
No. of observations	51014 (7389)
No. of unique observations	21093 (3190)
Redundancy	2.4 (2.3)
Completeness (%)	94.4 (98.2)
$\langle I/\sigma(I)\rangle$	7.3 (2.0)
$R_{\rm merge}$ (%)†	12.7 (46.8)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the ith observation of reflection hkl and $\langle I(hkl)\rangle$ is the weighted average intensity of i observations of reflection hkl.

1968) suggested that the asymmetric unit contained two IL-22–IL-22BP complexes with 57.5% solvent content ($V_{\rm M} = 2.89$ Å^3 Da^{-1}). The structure of the IL-22–IL-22BP complex was solved by the molecular-replacement technique using the atomic coordinates of the IL-22–IL-22R1 crystal structure (PDB code 3dlq; Bleicher *et al.*, 2008) as a search model. Prior to the rotation search, the search model was improved using *CHAINSAW* (Collaborative Computational Project, Number 4, 1994), which compares the chosen model and the given protein sequence, prunes nonconserved residues to the last common atom and retains conserved residues. This improved new model was used for molecular replacement with *Phaser* v.1.3.3 (McCoy *et al.*, 2007).

A rotation search conducted in the resolution range 22.6–3.1 Å yielded two clear top solutions with Z scores of 14.47 and 12.55 and with no other solution found over 75% of the top solution, suggesting the presence of two complexes per asymmetric unit (ASU), which is in agreement with the Matthews estimate. A translation function performed in the same resolution range also found two clear solutions (Z scores of 22.35 and 18.95), confirming the presence of two complexes in the ASU and defining the crystal space group as $P4_1$. Fixing the first solution, a search for a second molecule increased the Z score to 48.74. This solution, containing a dimer of the complex in the ASU, was used as the final MR solution. *Phaser* also reported no structural clashes between the molecules and its symmetry partners.

The first structural refinement cycle using the *PHENIX* (Afonine *et al.*, 2005) simulated-annealing procedure resulted in an $R_{\rm work}$ of 29.26% and an $R_{\rm free}$ of 34.6%. Further structural refinement is in progress.

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