

Crystallization and synchrotron X-ray diffraction studies of human interleukin-22

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Human interleukin-22, a novel member of the cytokine family, has been crystallized in hanging drops using the vapour-diffusion technique. Preliminary X-ray diffraction experiments using synchrotron radiation reveal that the protein crystallizes in space group $P2_12_12_1$, with unit-cell parameters $a = 55.44$, $b = 61.62$, $c = 73.43$ Å, and diffracts beyond 2.00 Å resolution.

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1. Introduction

Interleukin-22 (IL-22), also known as interleukin-10-related T-cell-derived inducible factor (IL-TIF), a protein which shares 22% identity with interleukin-10 (IL-10), has been recently identified in interleukin-9 (IL-9) induced murine T cells (Dumoutier, Louahed *et al.*, 2000) and subsequently in human cells (Dumoutier, Van Roost, Colau *et al.*, 2000) as a novel member of the cytokine family. *In vitro* experiments showed that expression of IL-22 is rapidly induced by IL-9 in T cells, with maximal levels reached in 1 h, and that this induction does not require protein synthesis as the upregulation of the IL-22 mRNA is not blocked by cycloheximide (Dumoutier, Louahed *et al.*, 2000). This new protein, like other cytokines, has an approximate size of 20 kDa and an N-terminal hydrophobic signal peptide. Human and mouse IL-22 (hIL-22 and mIL-22) share 79% amino-acid sequence identity and both have 179 amino-acid residues.

Cytokines exert their actions by binding to specific cell-surface receptors, which leads to the activation of cytokine-specific signal transduction pathways. Two distinct receptor chains from the class II cytokine receptor family have been identified as taking part in the IL-22-receptor complex (Xie *et al.*, 2000; Kotenko *et al.*, 2001). These receptors are the CRF2-9 (IL22-R) and the CRF2-4 (IL-10R2 or IL-10R β), the latter also being a functional component of the IL-10 signalling complex (Kotenko *et al.*, 1997). This is the first scientific observation within the class II cytokine receptor family of a receptor being utilized as a component of multiple distinct cytokine signalling complexes.

It has been demonstrated *in vivo* that mIL-22 expression is rapidly increased in several mice organs after lipopolysaccharide (LPS) injection, indicating that the role of IL-22 may not be restricted to the immune system and that it is also involved in inflam-

matory responses (Dumoutier, Van Roost, Colau *et al.*, 2000). The latter is corroborated by the fact that IL-22 stimulation of HepG2 human hepatoma cells up-regulated the production of acute phase reactants such as serum amyloid A, α 1-antichymotrypsin and haptoglobin (Dumoutier, Van Roost, Colau *et al.*, 2000). Moreover, there are data linking IL-22 to asthma and allergy owing to the probable involvement of IL-9 in these two pathologies (Temann *et al.*, 1998; McLane *et al.*, 1998; Levitt *et al.*, 1999). Other evidence for this linkage could be obtained at the DNA level. The hIL-22 gene is located on chromosome 12q (Dumoutier, Van Roost, Ameye *et al.*, 2000), where several loci potentially linked to asthma have been identified (Cookson, 2000).

In this paper, we describe the crystallization and results of preliminary X-ray diffraction studies of recombinant hIL-22 (rhIL-22) at 2.00 Å resolution.

2. Protein purification

Human IL-22 (corresponding to amino acids Gln29-Ile179; without the signal peptide) was cloned and expressed in *Escherichia coli* strain BL21 codon plus-(DE3)-RIL (Stratagene) as described in Dumoutier, Van Roost, Colau *et al.* (2000). Cells containing rhIL-22 were disrupted and inclusion bodies were collected by centrifugation. They were washed extensively first with 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, 1 mM DTT, 0.5% (w/v) deoxycholate (DOC) pH 8 and finally with the same buffer without detergent. Inclusion bodies were solubilized overnight in 8 M urea, 50 mM MES, 10 mM EDTA, 0.1 mM DTT pH 5.5. The rhIL-22 protein was refolded by direct dilution of the solubilized inclusion bodies in the following folding mixture: 100 μ g ml⁻¹ rhIL-22, 100 mM Tris-HCl, 2 mM EDTA, 0.5 M L-arginine, 1 mM reduced glutathione, 0.1 mM oxidized glutathione pH 8. The solution was incubated for 72 h. The folding

mixture was then concentrated by ultrafiltration in an Amicon chamber with a YM3 membrane before purification on a Superdex75 (Amersham Pharmacia Biotech) gel-filtration column. The protein was eluted with 25 mM MES, 150 mM NaCl pH 5.4. Recombinant human IL-22 peak fractions were concentrated to 5 mg ml⁻¹ with a YM3 Amicon membrane and desalted using a Hi-Prep 26/10 column (Amersham Pharmacia) with elution buffer containing 10 mM MES pH 5.4. The protein was concentrated again to 5 mg ml⁻¹ and lyophilized in 1 mg fractions.

3. Crystallization and data collection

Initial crystallization conditions were screened by the sparse-matrix method using the macromolecular crystallization reagent kits Crystal Screen I and II (Hampton Research) at 291 K. Small crystals were found in conditions number 18, 26 and 29 of the Crystal Screen I kit. In each trial, a hanging drop of 1 µl protein solution (10 mg ml⁻¹ in 20 mM MES buffer pH 5.4) was mixed with 1 µl precipitant solution and equilibrated against a reservoir containing 500 µl precipitant solution. Several attempts to improve crystal quality were performed, including pH and precipitant concentration refinement, detergent addition and macro-seeding. Well diffracting crystals were finally obtained using a precipitant solution consisting of 0.9 M sodium tartrate, Triton X-100 detergent and 0.1 M HEPES pH 7.5. Two synchrotron X-ray diffraction data sets, one native and one derivative, have been collected for this study. Both data sets were collected using a 345 mm MAR Research image-plate detector at the Brazilian National Synchrotron Light Laboratory protein crystallography beamline (Polikarpov, Perles *et al.*, 1997; Polikarpov, Oliva *et al.*, 1997) by the oscillation method at 100 K. The native crystal was immersed for 30 s in a cryocooling solution (precipitant solution with 15% ethylene glycol), mounted in a rayon loop and flash-cooled to 100 K in a cold nitrogen stream. An iodine derivative was prepared following the quick cryosoaking derivatization procedure (Dauter *et al.*, 2000; Nagem *et al.*, 2001). A single crystal was immersed for 3 min in a cryocooling solution (the same as used for

the native crystal) additionally containing 0.125 M sodium iodide and then frozen in a rayon loop in a cold nitrogen-gas stream (100 K). The synchrotron radiation wavelength was set to 1.54 Å to optimize the X-ray flux and to increase the anomalous signal for the iodine derivative. The initial images of each data set were subjected to the auto-indexing routine of *DENZO* (Otwinowski & Minor, 1997), which suggested a primitive orthorhombic cell to be the best solution. Following an optimum strategy of data collection suggested by the program *marHKL*, totals of 103 and 248° of native and iodine-derivative data were collected, respectively. The images were processed and scaled with the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997).

The iodine derivative, contrary to most derivatives prepared by traditional soaks, did not suffer a considerable loss of diffraction power, modification of unit-cell parameters or degradation during its preparation and has a great chance of being isomorphous (Table 1).

A search for additional heavy-atom derivatives and phase calculations using the SIRAS method are currently under way.

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Table 1

Native and iodine-derivative crystal data and data-collection statistics.

Values for the highest resolution shell are shown in parentheses.

	Native	Iodine derivative
Space group	<i>P</i> 2 ₁ 2 ₁ 2 ₁	<i>P</i> 2 ₁ 2 ₁ 2 ₁
Unit-cell parameters (Å)	<i>a</i> = 55.44, <i>b</i> = 61.62, <i>c</i> = 73.47	<i>a</i> = 56.05, <i>b</i> = 61.78, <i>c</i> = 73.63
Resolution (Å)	21.7–2.00 (2.05–2.00)	21.8–1.92 (1.96–1.92)
No. of reflections	61846	182876
No. of unique reflections†	16382	37777
<i>I</i> / <i>σ</i> (<i>I</i>)	14.5 (3.8)	13.4 (3.1)
Multiplicity	3.8 (3.4)	4.8 (4.3)
Completeness (%)	92.7 (82.3)	99.9 (99.7)
<i>R</i> _{merge} ‡ (%)	8.2 (35.0)	11.7 (43.9)
<i>R</i> _{int} § (%)		21.7
Data collected (°)	103	248
Cryoprotectant solution	Mother liquor, 15% ethylene glycol	Mother liquor, 15% ethylene glycol, 0.125 M NaI
Soaking time (s)	30	180

† Multiplicity of derivative and native data sets calculated with Friedel-related reflections treated separately and as equivalent, respectively.

‡ $R_{merge} = \sum_{hkl} |I_{hkl} - \langle I_{hkl} \rangle| / \sum_{hkl} I_{hkl}$. § $R_{int} = \sum_{hkl} |F_{PH} - F_P| / \sum_{hkl} F_P$.

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