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Purification, crystallization and preliminary crystallographic studies of the complex of interferon-λ1 with its receptor

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Human interferon-λ1 (IFN-$\lambda1_{Ins}$) and the extracellular domain of interferon-λ1 receptor (IFN-λ1R1) were expressed in *Drosophila* S2 cells and purified to homogeneity. Both IFN-$\lambda1_{Ins}$ and interferon-λ1 produced from *Escherichia coli* (IFN-$\lambda1_{Bac}$) were coupled with IFN-λ1R1 at room temperature and the complexes were purified by gel filtration. Both complexes were crystallized; the crystals were flash-frozen at 100 K and diffraction data were collected to 2.16 and 2.1 Å, respectively. Although the IFN-$\lambda1_{Bac}$–IFN-λ1R1 and IFN-$\lambda1_{Ins}$–IFN-λ1R1 complexes differed only in the nature of the expression system used for the ligand, their crystallization conditions and crystal forms were quite different. A search for heavy-atom derivatives as well as molecular-replacement trials are in progress.

1. Introduction

Interferon-λ1, interferon-λ2 and interferon-λ3 (IFN-λ1, IFN-λ2 and IFN-λ3) belong to a recently discovered group of type III interferons (Kotenko *et al.*, 2003) and are also known as interleukin-29 (IL-29), IL-28A and IL-28B, respectively (Sheppard *et al.*, 2003). IFN-λs, together with the classical type I IFNs IFN-α and IFN-β, serve as master regulators of a multifaceted antiviral response (Meager *et al.*, 2005). Both type I and type III IFNs are coproduced by various nucleated cells in response to live viral infections and to a variety of toll-like receptor (TLR) stimuli (lipopolysaccharides, poly-I:C, bacterial/viral DNA; Kotenko & Langer, 2004). Moreover, both types of IFNs activate the same signal transduction pathways, including the formation of the same interferon-stimulated gene factor 3 transcription complex (Kotenko *et al.*, 2003), and induce the expression of the same set of genes (Doyle *et al.*, 2006; Marcello *et al.*, 2006; Zhou *et al.*, 2007); they therefore have very similar biological activities that include a strong intrinsic antiviral activity (Kotenko & Langer, 2004). However, whereas type I IFNs are able to activate a potent antiviral state in a wide variety of cells (Meager *et al.*, 2005; Pestka *et al.*, 2004), type III IFNs are primarily active on epithelial cells (Lasfar *et al.*, 2006; Sommereyns & Michiels, 2006). The cell type-selective action of type III IFNs is possible because each type of IFN engages its own unique receptor complex for signaling and because of the distinct expression pattern of IFN receptors. Whereas type I IFNs signal through a common cellular IFN-α/β receptor complex composed of two unique subunits, IFN-αR1 and IFN-αR2, which are ubiquitously expressed (Cutrone & Langer, 2001; Pestka *et al.*, 2004), type III IFNs signal through an IFN-λ receptor complex which consists of a unique IFN-λR1 chain and a shared IL-10R2 chain that is also the second subunit of the IL-10, IL-22 and IL-26 receptor complexes (Kotenko & Langer, 2004). In contrast to the IFN-α/β receptor subunits and the IL-10R2 chain, which are ubiquitously expressed, the IFN-λR1 chain is expressed primarily by epithelial cells and dendritic cells, restricting the action of type III IFNs to epithelial cells (Lasfar *et al.*, 2006; Sommereyns & Michiels, 2006).

Type III IFNs demonstrate the same, albeit very low, percentage amino-acid similarity to both type I IFNs and IL-10-related cytokines

Table 1
Data-collection statistics.

Values in parentheses are for the highest resolution shell.

Complex	IFN-λ1$_{Bac}$–IFN-λ1R1	IFN-λ1$_{Ins}$–IFN-λ1R1
Space group	$P2_12_12$	$P2_12_12_1$
Unit-cell parameters (Å)	a = 130.2, b = 65.4, c = 73.2	a = 65.0, b = 85.8, c = 116.5
Resolution (Å)	2.16 (2.24–2.16)	2.1 (2.18–2.10)
Measured reflections	122399	258890
Unique reflections	33364	37819 (3044)
Completeness (%)	97.4 (81.7)	97.1 (79.6)
Redundancy	3.7 (2.7)	6.8 (4.2)
R_{merge}†	0.057 (0.71)	0.07 (0.84)
$I/\sigma(I)$	16.8 (1.9)	23.6 (2.2)

† $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$.

(Kotenko & Langer, 2004; Pestka *et al.*, 2004). Their receptor subunits are structurally related, share a low degree of amino-acid similarity in their extracellular domains and belong to the family of class II cytokine receptors (Kotenko, 2007; Langer, 2007). Here, we report the expression, purification and preliminary X-ray analysis of IFN-λ1 expressed in *Escherichia coli* and *Drosophila* S2 cells complexed with its receptor IFN-λ1R1.

2. Materials and methods

2.1. Protein production and purification

A cDNA fragment encoding the mature human IFN-λ1 protein (amino acids 1–181) was excised from plasmid pEF-FL-IFN-λ1 (Kotenko *et al.*, 2003; Sheppard *et al.*, 2003) with the use of *Bam*HI and *Eco*RI restriction endonucleases and recloned into the corresponding sites of a modified pMT/BiP/V5-His vector (Invitrogen) carrying the BiP *Drosophila* signal peptide followed by an N-terminal 6×His tag. A cDNA fragment encoding mature human IFN-λ1R1 protein (amino acids 1–208; Kotenko *et al.*, 2003; Sheppard *et al.*, 2003) was amplified with primers 12-3 (5′-GCCGGATCCCCGTCTGGCCCCTCCCCAGAA-3′) and 12z (5′-GCGACTAGTCCCAGAGCTACCACCAGAACTCCCGCTAGCGTTGGCTTCTGGGACCTCCAG-3′), digested with *Bam*HI and *Spe*I restriction endonucleases and cloned into the *Bam*HI and *Nhe*I sites of a modified pMT/BiP/V5-His vector (Invitrogen) carrying the BiP *Drosophila* signal peptide and a C-terminal 6×His tag. The nucleotide sequences of the modified regions within the plasmids were verified in their entirety by sequencing.

The engineered sequences were transfected into *Drosophila* S2 cells using the cationic lipid Maxfect (Molecular Research Laboratories Inc, Herndon, Virginia, USA). Blasticidin S plasmid (Invitrogen) was cotransfected into the cells to allow drug selection. Media containing antibiotics were changed every 72 h until the amount of antibiotic resistant cells in tissue-culture plates and flasks reached approximately 1×10^8 ml^{-1}. Cells were then propagated without antibiotics as spinner cultures at densities of 1×10^6 and 1×10^7 ml^{-1}. As the proteins were secreted into the media, they were purified directly from it by copper affinity chromatography (GE Healthcare Fast Flow Chelating Sepharose). Briefly, an affinity column (Lehr *et al.*, 2000) was equilibrated with deionized water and filtered (0.45 µm pore size, Nalgene) medium was then loaded onto the column at a flow rate of 10 ml min^{-1}. Since the cells were induced with 500–750 µ*M* $CuSO_4$, no prior charging of the chelating Sepharose with copper was required. The column was extensively washed with 50 m*M* HEPES, 0.5 *M* NaCl pH 7.0 to remove non-specifically bound proteins. The process was monitored by UV absorbance at 280 nm. The second washing step used the buffer described above plus 55 m*M* imidazole and the protein was subsequently eluted with 250 m*M* imidazole. Proteins were concentrated (Amicon-15, 10K) and applied onto a Superdex-75 HiLoad 16/60 column (Pharmacia, GE Healthcare) pre-equilibrated with 50 m*M* HEPES, 200 m*M* NaCl pH 7.0 at a flow rate of 0.33 ml min^{-1} and collected as 1 ml fractions. A STAT activation electrophoretic mobility-shift assay showed that IFN-λ1$_{Ins}$ was fully active in comparison with IFN-λ1 produced by COS cells. IFN-λ1$_{Bac}$ was purchased from Preprotech. The activity of IFN-λ1R1 was checked by its ability to form binary complexes.

2.2. Ligand–receptor complex formation

The formation of complexes of IFN-λ1R1 with IFN-λ1, the latter of which was expressed either in bacteria (IFN-λ1$_{Bac}$) or in insect cells (IFN-λ1$_{Ins}$), was performed using the same protocol. The ligand and the receptor were mixed together in a 1:1 molar ratio in 50 m*M* HEPES, 200 m*M* NaCl pH 7.0 and left for about 2 h at room temperature. Subsequently, the material was centrifuged for about 15 min and applied onto a size-exclusion column. The flow rate and fraction size were the same as during the purification of the separate components. Fractions corresponding to the complex were pooled and concentrated to about 6 mg ml^{-1}. A typical batch gave about 3 mg of pure complex.

2.3. Crystallization

All crystallization trials were set up at room temperature. Initial experiments were carried out using a Phoenix crystallization robotic system (Art Robbins Instruments, Sunnyvale, California, USA), employing the sitting-drop method. CrystalQuick 96, three-drop well crystallization plates and Index (Hampton Research), PEG and pHClear (Nextal Biotechnologies) screens were used in the initial trials. The drop size in these experiments was 0.6 ml. Crystals suitable for X-ray analysis were subsequently obtained by the hanging-drop vapor-diffusion method using Qiagen EasyXtalTools 24-well plates. The well volume was 0.75 ml and the drop contained 2 µl protein solution and 1 µl well solution. 48 and 96 optimization droplets were set up to refine the final conditions. In the case of the IFN-λ1$_{Bac}$–IFN-λ1R1 complex, the crystallization conditions were 17% PEG 3350 and 100 m*M* HEPES pH 7.8. In the case of the IFN-λ1$_{Ins}$–IFN-λ1R1 complex the conditions were 20% MPEG 2000, 100 m*M* Tris–HCl pH 7.9 and 200 m*M* trimethylamine *N*-oxide dehydrate. In

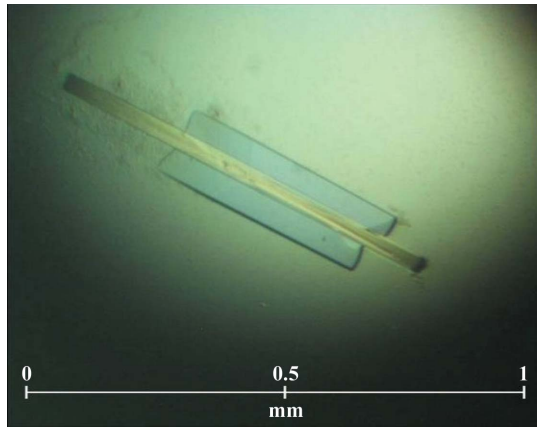

Figure 1
Crystals of IFN-λ1$_{Ins}$–IFN-λ1R1 grown by the hanging-drop vapor-diffusion method after 4 d.

both cases crystals appeared the next day and reached their final size in 4 d. Although the crystals were orthorhombic in both cases, with the unit cell containing one complex in the asymmetric unit, they were not isomorphous. The IFN-λ1$_{Bac}$–IFN-λ1R1 complex crystallized in space group $P2_12_12$, with unit-cell parameters a = 130.2, b = 65.4, c = 73.2 Å, whereas the IFN-λ1$_{Ins}$–IFN-λ1R1 complex belonged to space group $P2_12_12_1$, with unit-cell parameters a = 65.0, b = 85.8, c = 116.5 Å (Fig. 1).

2.4. Data collection and analysis

Diffraction data were collected on the Southeast Regional Collaborative Access Team (SER-CAT) beamline ID-22 at the Advanced Photon Source, Argonne National Laboratory using a MAR 300 CCD detector. Crystals were transferred to a cryprotectant solution (20% glycerol mixed with 80% well solution) and immediately frozen in a nitrogen stream at 100 K. The total rotation of the crystal around the spindle axis during data collection was 190° and 360° for IFN-λ1$_{Bac}$–IFN-λ1R1 and IFN-λ1$_{Ins}$–IFN-λ1R1, respectively. Both crystal forms diffracted to about 2.1 Å resolution (Table 1). The *DENZO*/*SCALEPACK* programs (Minor *et al.*, 2006; Otwinowski *et al.*, 2003; Otwinowski & Minor, 1997) were used to process the data and the final statistics are listed in Table 1.

3. Results and discussion

Human IFN-λ1$_{Ins}$ and IFN-λ1R1 were expressed in *Drosophila* Schneider 2 (S2) cells and purified to homogeneity. During expression both properly folded mature proteins were secreted into conditioned media, allowing us to use a two-step purification protocol consisting of Cu^{2+}-affinity chromatography followed by size-exclusion chromatography. IFN-λ1$_{bac}$–IFN-λ1R1 and IFN-λ1$_{Ins}$–IFN-λ1R1 complexes were prepared at room temperature and purified from unbound components by gel filtration, following a protocol similar to that published for the complexes of IL-19 and IL-20 with their receptor IL-20R1 (Pletnev *et al.*, 2003). Both complexes were crystallized; the crystals were flash-frozen at 100 K and diffraction data were collected to 2.16 and 2.1 Å resolution, respectively (Table 1). Despite the fact that the two complexes differed only in the nature of the host in which the ligands were expressed, the crystallization conditions and crystal forms were quite different. Likely reasons for this difference could be either the presence of an N-glycosylation site in the *Drosophila*-expressed ligand at Asn46 (mature protein-numbering scheme) or the presence of a 6×His tag at the N-terminus of IFN-λ1$_{Ins}$. However, the final answer will have to await the determination of both structures. A heavy-atom derivative search and molecular-replacement trials are in progress.

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