Expression, purification, and co-crystallization of IRF-I bound to the interferon-β element PRD I

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Abstract Interferon regulatory factor 1 (IRF-1) is an essential factor involved in the regulation of type I interferon (IFN) and IFN-inducible genes. The protein consists of 329 amino acids that are highly conserved from mouse to human. Similar to other transcription factors, the protein is modular in nature with a basic N-terminal region involved in DNA binding and an acidic C-terminal region required for activation. We report here the expression, purification and co-crystallization of the minimal N-terminal region of IRF-1 involved in DNA binding (amino acids 1–113) with a 13 bp DNA fragment from the IFN-β promoter. The crystals diffract to at least 3.0 Å in resolution and belong to space group R3 with unit cell parameters of $a = b = 84.8$ Å, $c = 203.7$ Å.

Key words: X-ray crystallography; Interferon; Transcription factor; DNA-binding protein

1. Introduction

Upon viral infection, a cascade of signal transduction mechanisms is set in motion in the cell that ultimately bring about the production of type I interferon molecules (IFN-α and IFN-β). Regulation of this process at the transcriptional level is modulated by a panoply of protein factors that bind to their promoter/enhancer sites and act in a concerted mode to activate or repress gene expression [1,2]. Interferon regulatory factor 1 (IRF-1) is an essential player in the activation of type I *IFN* genes and several IFN-inducible genes [1,2]. More recently, IRF-1 has been shown to be involved in processes such as cell growth, apoptosis, regulation of the nitric oxide synthase (*iNOS*) gene, and resistance to bacterial infection [3–6]. Molecular cloning and mutational analysis of IRF-1 have shown that the N-terminal region is responsible for DNA binding while the acidic C-terminal region is required for activation [7]. Sequence homology analysis reveals that IRF-1 belongs to a family of IFN regulatory factors that includes IRF-2 [8], and the transcription factors ISGF3-γ and ICSBP that are involved in the JAK/STAT signalling pathway [9,10]. IRF-1 and IRF-2 compete for the same DNA sites (PRDI and PRDIII) on the IFN-β promoter, with IRF-1 acting as a transcriptional activator and IRF-2 acting as a transcriptional repressor [11]. To date, the only structural information relevant to IRF-1 is the secondary structure assignment of IRF-2 by NMR [12]. Here we report the first co-crystals of a member of the IRF-1 family and its target DNA site.

2. Materials and methods

We first undertook a proteolytic study to characterize the DNA binding domain of IRF-1. Partially purified recombinant full-length IRF-1 was digested with the non-specific protease papain. A time-course reaction was carried out at room temperature with two different IRF-1/papain ratios. A stable domain of ~14 kDa was found to be the predominant species in the digestion reaction. Gel retardation analysis showed that this fragment bound to DNA as equally well as the intact protein. The proteolytic fragment was purified by gel filtration (Superdex 75) and subjected to NH_2-terminal sequencing and mass spectrometry. Results showed that the proteolytic fragment encompassed the region between amino acids 6–114. Based on these results, we subcloned the IRF-1 DNA-binding region (amino acids 1–113) into the vector pET-3a (Novagen) and expressed it in *E. coli* as follows: A colony of BL21(DE3) pLysS *E. coli* cells freshly transformed with the plasmid pET-3IRF1 was used to inoculate 20 ml of 2×YT media containing 200 μg/ml of ampicillin. The cells were grown overnight at 37°C, and a 2 ml aliquot of the culture was used to inoculate 1 l of 2×YT media containing 200 mg of ampicillin. Cells were grown at 37°C to an OD_{600} of 0.6 and then induced with the addition of IPTG to a final concentration of 0.4 mM. After 4 h, the cells were harvested by centrifugation at 5000 rpm for 10 min and stored at −70°C. The protein expresses mostly in inclusion bodies.

To purify the protein, frozen cells from the 1 l culture were resuspended in 150 ml of buffer HK500 (50 mM HEPES, pH 7.5, 500 mM KCl, 10% glycerol) containing 0.1% NP40, 1 mM EDTA, and 0.2 mM PMSF. The suspension was sonicated for 2 min and then centrifuged for 40 min at 15000 rpm. The insoluble fraction containing IRF-1(1–113) was washed thoroughly by four consecutive cycles of resuspension in HK500, sonication, and centrifugation (15 min at 15000 rpm). After the final centrifugation the pellet was solubilized in buffer G (6 M guanidine hydrochloride (Gu·HCL), 50 mM HEPES, 500 mM KCl, 10 mM DTT) and dialyzed for 12 h against each of the following solutions: HK500 + 2 M Gu·HCL, HK500 + 1 MGu·HCL, HK500 + 0.5 M Gu·HCl. Final dialysis was against HK500 containing 10 mM DTT. The protein was ~90% pure at this stage. For the final purification, the protein was concentrated down to 10 ml and loaded onto a Superdex 75 column previously equilibrated with buffer HAA500 (50 mM HEPES, 500 mM ammonium acetate, 10% glycerol, 10 mM DTT, pH 6.5). A single liter of bacterial cells yielded ~40 mg of pure protein. The DNA-binding activity of the refolded protein was comparable to that of the native protein. For crystallization the protein was concentrated to 10–13 mg/ml by ultrafiltration (Amicon), aliquoted, and stored at −70°C.

3. Results and discussion

For co-crystallization studies, we synthesized and purified a series of DNA fragments (10–18 bp) based on the PRDI sequence from the IFN-β promoter. Of these, only the blunt-ended 13-mer containing the exact PRDI sequence was found

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5'-GAGAAGTGAAAGT-3'
3'-CTCTTCACTTTCA-5'
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Fig. 1. The 13-mer oligomer used in IRF-1/DNA co-crystallization. The sequence matches the PRDI site from the IFN-β promoter.

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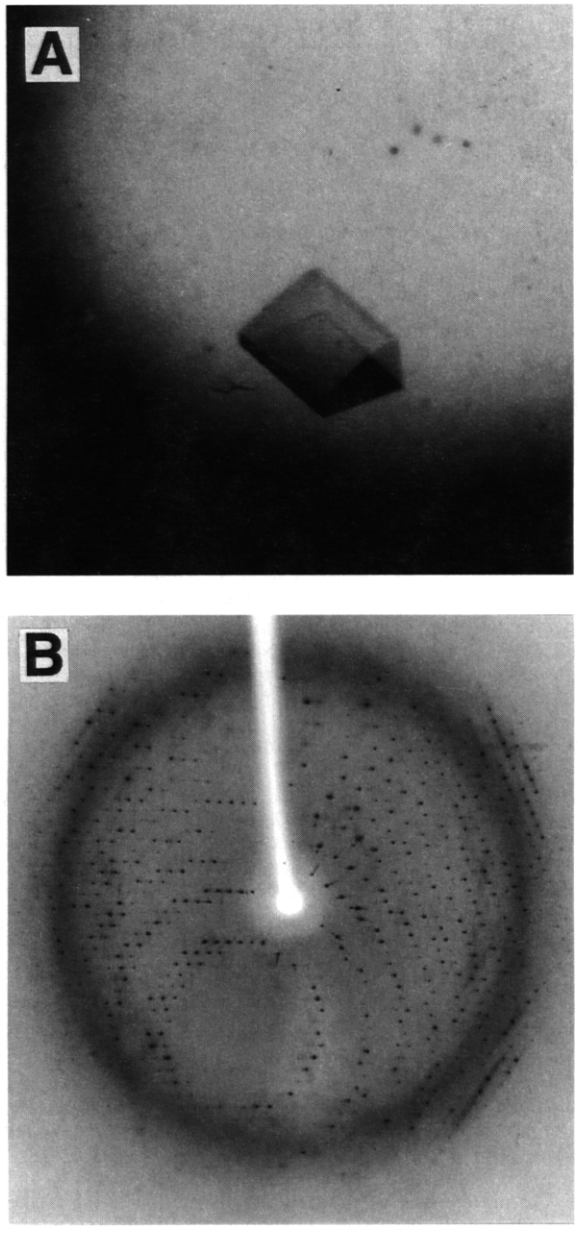

Fig. 2. A: A co-crystal measuring ~0.4×0.3×0.1 mm of IRF-1/PRDI complex. B: Diffraction from a frozen co-crystal recorded with a Rigaku RU200 rotating anode X-ray generator. The image corresponds to a 2° oscillation and shows diffraction to at least 3 Å resolution. The streaks corresponding to 'DNA reflections' can be seen in the picture.

to produce co-crystals (Fig. 1). Initial co-crystallization trials were performed by the sparse matrix method [13], using hanging drops. Co-crystallization trials at room temperature produced only microcrystals. However, at 4°C we were able to identify conditions that yielded large needles. These conditions were eventually refined to produce large, chunky co-crystals, measuring up to 0.4×0.3×0.1 mm (Fig. 2A). The best co-crystals begin to appear after a few hours and grow to a full size within 3 days from 18 to 22% polyethylene glycol 8000 (PEG 8K) solutions containing 300 mM ammonium acetate (pH 6.5). The presence of protein and DNA in the co-crystals was confirmed by gel electrophoresis. For diffraction analysis, the co-crystals were transferred in a stepwise manner (using 5% glycerol increments) to a final cryoprotectant solution containing 25% PEG 8K, 20% glycerol, and 300 mM ammonium acetate.

The co-crystals diffract to 3.0 Å resolution on an R-axis II imaging plate area detector mounted on a Rigaku rotating anode X-ray source (100 mA and 50 kV) (Fig. 2B). Using the HKL programs (HKL Research), the co-crystals were found to belong to space group R3 with unit cell dimensions of $a = 84.8$ Å, $b = 84.8$ Å, $c = 203.7$ Å, $\alpha = 90°$, $\beta = 90°$, $\gamma = 120°$. Surprisingly, synchrotron radiation did not extend the resolution of the co-crystals. Assuming a model in which one IRF-1 molecule is bound to the 13-bp DNA fragment, a V_m calculation suggests that there are two such complexes in the crystallographic asymmetric unit. This gives a V_m value, 3.3 Å^3/Da, which is within the range of values, 1.68–3.53 Å^3/Da, observed with protein crystals [14,15]. We also measured multiwavelength anomalous diffraction (MAD) data from a brominated derivative of the IRF-1/PRDI complex. The derivative was prepared by substituting bromouracils for five thymine residues within the PRDI sequence. The MAD data were measured from a single frozen cocrystal (−162°C) at Cornell High Energy Synchrotron Source (CHESS). The data were recorded using the 2k×2k charge coupled device (CCD) detector at beamline F2. The MAD data are 99% complete to 3 Å resolution and have R_{syms} in the range of 6.1–6.9%. The structure of IRF-1/DNA complex promises to provide a framework for understanding how IRF-1 family members recognize DNA in order to regulate the interferon system.

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