Determining the Structure of Interferon Regulatory Factor 3 Using X-Ray Crystallograph

Student Researcher: Hyowoun Jyung
Mentor: Carlos Escalente, PhD

Interferon Regulatory Factor 3 (IRF-3) is a primary activator of interferon genes and other genes that are vital in immune response. The protein consists of 427 amino acids. The N-terminal region is involved in DNA binding and the C-terminal region is activated upon viral infection. The focus of this study was on the N-terminal region, also known as the DNA binding domain (DBD), and interacts with DNA to activate the gene transcription. IRF-3 at this region was crystallized, with large adequate crystals formed in 11% PEG 1K, 200 nM ZnAc, and 100nM NaAc pH 5. When 2μL of 30% 1, 5 diaminopentane diHCl was added to the protein, the crystal improved greatly. To determine the structure using multi-wavelength anomalous diffraction (MAD), a crystal of a mutated protein is needed so that its diffraction data can be compared with that of the wild-type protein. The two-point mutations on IRF-3 to include two selenium elements were created using Polymerase Chain Reaction (PCR). Once this mutated protein was crystallized, its diffraction data was collected and provided the three-dimensional electron density map and ultimately the structure of IRF-3. Further studies in the mechanism and the structure of IRF-3 promise pharmaceutical application as well as gene therapy for improved treatment of numerous diseases including type 1 diabetes, multiple sclerosis, and certain cancers.

Procedure

We formed IRF-3 using DNA recombinant technique and purified the protein using an HPLC machine. After trying hundreds of different combinations of solutions, the protein was crystallized using hanging drop method. Large, adequate crystals were formed in 11% PEG 1K, 200 μL ZnAc, and 100 NaAc pH 5. Screens 1, 2, and 3 of additives were tried in order to obtain sim-

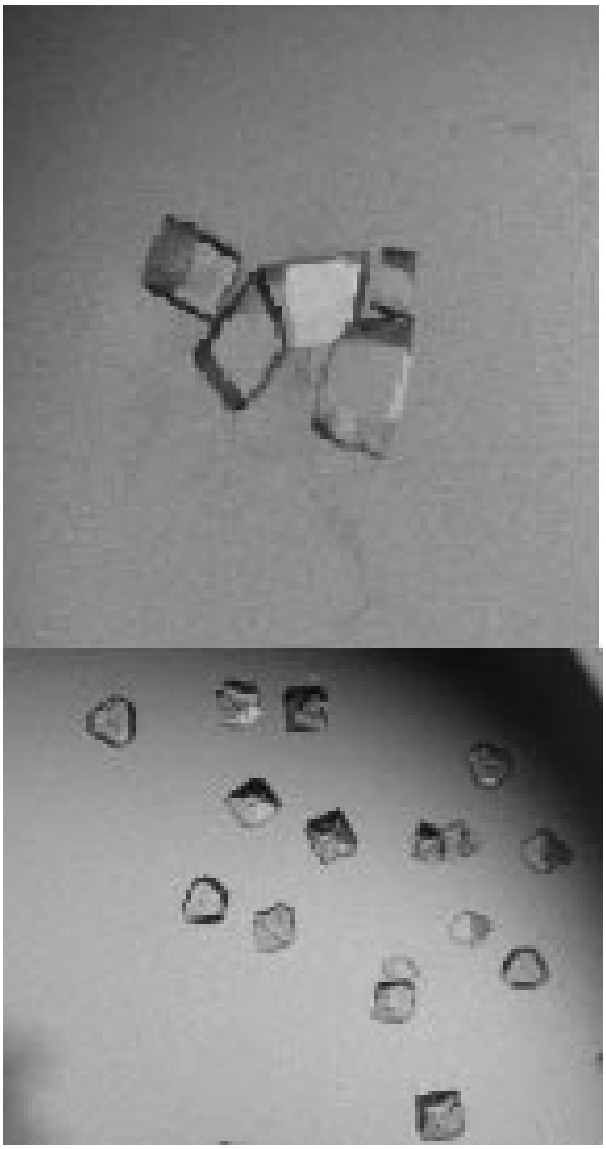

Fig 1. Large adequate crystals formed in 11% PEG 1K, 200 μL ZnAc, and 100 NaAc pH 5

From the Mount Sinai School of Medicine; Williston Park, New York.

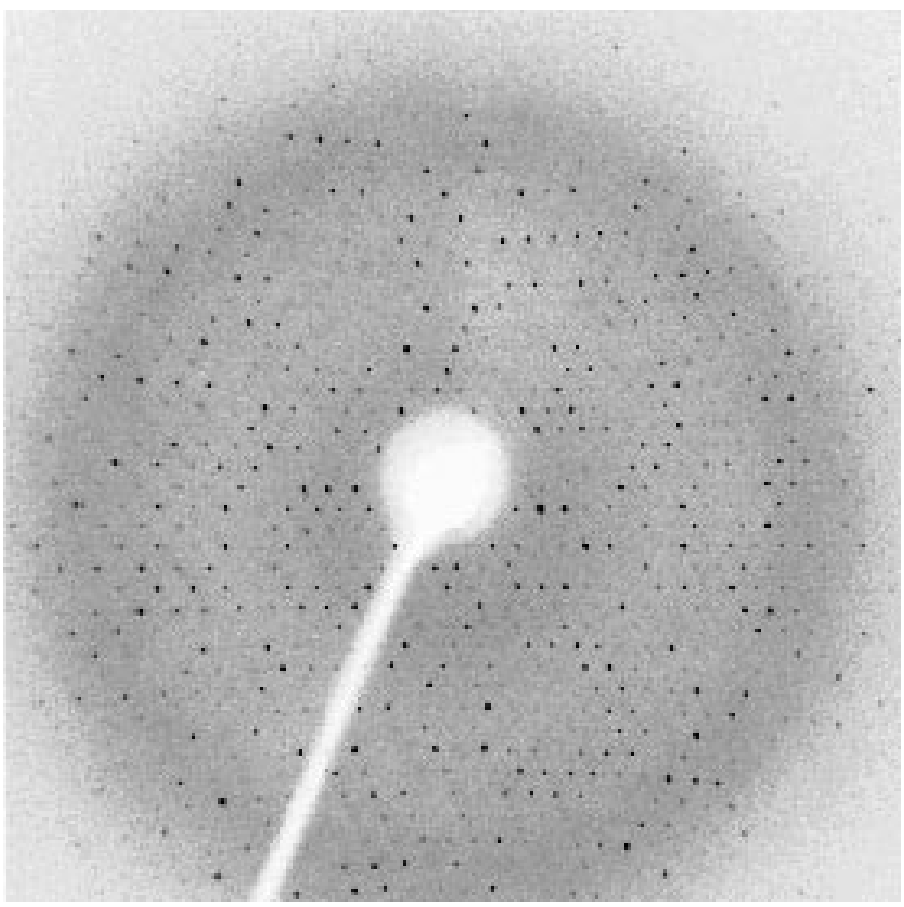

Fig 2. X-ray diffraction data

ilar crystals. 0.5 μL of additives were added to 2 μL of IRF-3 DBD and 2 μL of the aforementioned optimum solution. The additive, 30% 1, 5 diaminopentane diHCl, resulted in medium-sized bipyramidal-shaped crystals, as well as different shapes of large crystals adequate for diffraction. When the crystal was placed between the x-ray generator and the area detector screen, nitrogen gas at 109 Kelvin was directed at the crystal to freeze it. However, before these crystals are placed, different cryo-protectant series were tried in order to prevent hexagonal ice formation around the crystals and to maintain the proteins in order. The first cryo-protectant solution tried was a PEG 1k solution similar to the mother solution. This first series was as follows: 11%, 20%, 25%, 30% PEG 1K with 300 $ZnCl_2$ and 100 NaAc. A drop of the solutions was put on a cover slip and a crystal was put in each for one minute using a micro loop. No noticeable change was found in the crystal; however, the crystal did not diffract. The next series was carried using glycerol. Each solution was 11% PEG 1K and contained 5%, 10%, 20%, and 30% glycerol. The crystal, as soon as it was placed in glycerol, was distorted greatly. Before the third trial using ethylenglycol, some of the cover slips containing crystals were put into a tray of 30% PEG 1K overnight. These crystals diffracted well which meant that the right cryo-protectant condition was no longer needed as one can use this method to stabilize the crystal before freezing. One large crystal in particular diffracted the best. This IRF-3 DBD crystal was a primitive hexagonal as determined by the area detector and the computer program. With this data, one needs a diffraction data of a mutated IRF-3 crystal to solve its structure using MAD phasing method.

In order to obtain the mutated protein, PCR (polymerase chain reaction) was used to produce the mutated IRF-3 gene, which encodes for substitution of methylene for two of the leucines in wildtype IRF-3 gene. It was conformed by the gel electrophoresis that the desired gene was produced.

RESULTS

Large adequate crystals were formed in 11% PEG 1K, 200 μL ZnAc, and 100 NaAc pH 5 as seen in Figure 1. Screens 1, 2, and 3 of additives were tried in order to obtain similar crystals. 0.5 μL of additives were added to 2 μL of IRF-3 dbd and 2 μL of the aforementioned optimum solution. Although the additives such as 100 mM calcium chloride dihydrate, Jeffamine, and Yttrium Cl hexahydrate resulted in some crystals, 30% 1, 5 diaminopentane diHCl resulted in the most adequate crystals for diffraction. Several of these crystals have been used to collect x-ray diffraction data at the lab. One of the data is shown in Figure 2.

DISCUSSION AND CONCLUSION

It is critical to understand the structure and function of IRF-3 in order to elucidate the intricate mechanism of the immune system. In addition, there are numerous diseases that are closely linked to the malfunction of IFN stimulation. They include viral diseases such as Hepatitis and autoimmune diseases such as multiple sclerosis and insulin dependent diabetes mellitus (IDDM).

Although some of the diseases are already being treated with IFN injection, the treatment is costly and it has several

side effects. Studies on the structure and function of IRF transcription factors can lead to pharmaceutical applications of novel means to stimulate IFNs to circumvent the disadvantages of IFN injection. In addition, recent studies on IRF-3 reveal its role in cancer. IRF-3, by initiating innate immune response, can prevent retroviral oncogenesis. Additional studies demonstrate IRF-3 as an immunomodulatory factor, cell growth regulator, tumor suppressor, and angiogenesis inhibitor, suggesting novel means to study and treat cancer. In fact, a study shows that tumor growth could be inhibited in IRF-3 transduced B16 melanoma tumor cells *in vivo,* providing IRF-3 as a novel target of gene therapy. In this study, the substitution of serine and theonine residues in the amino acid 396–405 region of IRF-3 created an active form of IRF-3, which was able to stimulate gene expression without virus induction. This study demonstrates IRF-3 as a potent antiviral and an immunomodulatory factor.

Further studies in the mechanism of IFN and the structure of the proteins involved in the gene expression of IFN promise pharmaceutical application as well as gene therapy for the better treatment of these diseases. One of the future goals is to circumvent the disadvantages of IFN injection. Understanding the mechanism of IRF can lead us to program cells to produce IFN rather than injecting them. In addition, designing drugs that mimic IRF can be a novel strategy to regulate IFN transcription. Possessing promises to treat numerous diseases, further studies on IRFs should be continued.