Expression and Purification of Human IL-7 to Study its Immunological Properties

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Introduction

Interleukin 7 (IL-7) is a cytokine essential for T-cell survival. However, if the amount of signaling isn't properly regulated, serious malfunctions may occur including leukemias, lymphomas, and autoimmune diseases such as multiple sclerosis. By looking at the protein structure using X-ray crystallography, we can gauge a better idea of how it functions by analyzing the complex through which it signals. Before crystallization, the complex components must be purified. My role was to express and purify human interleukin 7 (hIL-7) from bacterial cells.

Background

For signaling to occur, the ternary complex must form with IL-7 and two cell surface receptors: IL-7Rα and common gamma-chain (γc), as shown in Figure 1. The goal for my purified hIL-7 will be to use it to help solve crystal structure of the activating signaling complex of IL-7 with IL-7Rα and γc.

The main part of the purification process consists of isolating hIL-7 from the other proteins. To accomplish this, it's important to consider its properties. hIL-7 is a positively-charged protein with a 17,563 Da molecular weight. The cation exchange and size exclusion chromatography columns target these properties using a resin that binds to protein based on its charge and separates based on size respectively.

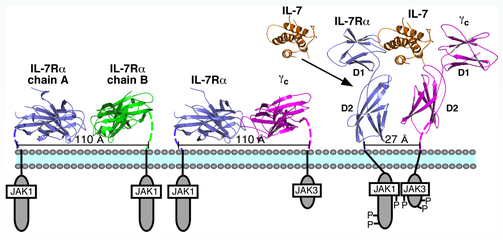

Figure 1. Ternary complex of IL-7, IL-7Rα, and γc. Without all three components, the JAK-STAT signaling pathway cannot be activated.

Methods

The E. coli cell line BL21(DE3) Codon+ RP was transformed with DNA of hIL-7 in the pET-15b expression vector. After inoculating the cells and growing them to optimal density, IPTG was used to induce protein production expression, which formed insoluble inclusion bodies. The bacteria were lysed using lysozyme and sonication, and a protease-inhibitors added to prevent random unwanted proteolysis. The subsequent steps consisted of inclusion body washing, rinsing, denaturing the hIL-7 inclusion bodies, and finally refolding. The hIL-7 was purified to homogeneity by cross-flow filtration (10 kDa MWCO filter), a cation exchange chromatography column, and finally by passage over a size exclusion chromatography column.

Data & Figures

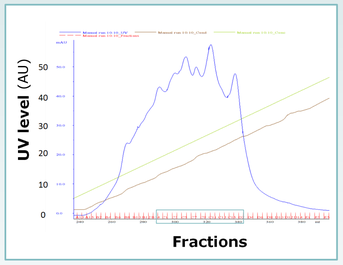

Figure 2. Cation exchange chromatogram from resS column. The spike in UV indicates protein unbinding from the resin. The fractions within the rectangle on the x-axis contain hIL-7. The green and brown lines show an increase in conductivity and concentration as Buffer B is gradually added to release the protein using salt.

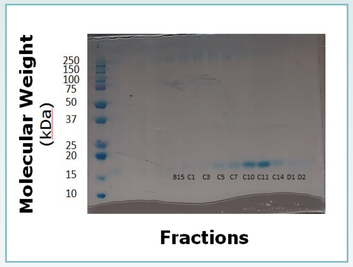

Figure 3. SDS page gel electrophoresis results after cation exchange. Fractions B15, C1, C3, C5, C7, C10, C11, C14, D1, and D2 were visible so B15 through D2 were chosen for spin concentration.

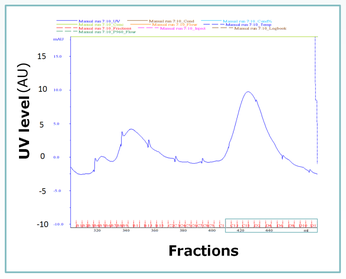

Figure 4. Size exclusion chromatogram from S200 column. The spike in UV indicates protein unbinding from the resin. The fractions within the rectangle on the x-axis contain hIL-7.

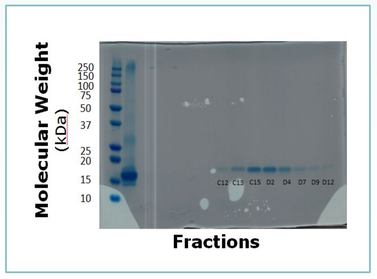

Figure 5. SDS page gel electrophoresis results after size exclusion. Fractions C12, C13, C15, D2, D4, D7, D9, and D12 were visible, so C12 through D12 were chosen for spin concentration.

Results

The cation exchange chromatogram is shown by Figure 2. In Figure 3, a SDS page gel, which was run based off the chromatogram, showed that fractions B15 through D2 contained hIL-7. Figure 4 displays the size exclusion chromatogram. This is accompanied by Figure 5 with the gel results showing that fractions C12 through D12 contained hIL-7. The purified protein was obtained once the S200 fractions were concentrated down to 7 mL.

The final yield was about 1.3 mg for 2 liters of culture with a protein concentration of 10.7 μM. Surface plasmon resonance (SPR) spectroscopy will be used to measure the binding affinity between my purified hIL-7 and the IL-7Rα. This will confirm that the hIL-7 is properly refolded and functional.

Future Steps

Currently, I'm running two new purifications alongside each other. I'm comparing the protein yield using E. coli optimized DNA for hIL-7 versus the non-optimized hIL-7 from a cDNA library. If the use of codon optimization improves the protein yields relative to the cDNA gene, the codon optimized hIL-7 gene will be used from now on and will improve the efficiency of this protocol.

Codon optimization works by using different codons for the same amino acids based on species' biological preferences. For example, humans prefer high amounts of guanine and cytosine nucleotides. However, E. coli do not, and they don't have the tRNA to translate so many G- and C-rich codons. Using preferred codons improves transcription efficiency and, therefore, should increase protein expression.

IL-7 has vast potential applications, including gene therapy to cure the many illnesses it is associated with. It may also be used to help rebuild the immune system of recovering chemotherapy patients.