Optimized Purification and Characterization of Recombinant Human Interleukin-2 (Rhil-2) Using a His-Mbp Fusion System

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Abstract:Recombinant human interleukin-2 (rhIL-2) is a key cytokine for cancer immunotherapy, but it has the propensity to express as insoluble inclusion bodies in Escherichia coli as it is hydrophobic. In this study, the soluble expression and purification of rhIL-2 was maximized in a His-MBP fusion system in the E. coli Origami B (DE3) host strain that supports disulfide bond formation. The fusion protein was overexpressed in a soluble state, processed using immobilized metal ion affinity chromatography (IMAC-Ni) and His-tagged TEV protease cleavage. Ni-NTA flow-through chromatography yielded rhIL-2 to >95% homogeneity. High-performance size exclusion chromatography (HP-SEC) confirmed the presence of monomeric and aggregated states, while bioactivity assays demonstrated the biologically active nature of the purified rhIL-2 with an EC50 value (3.13 ng/mL) comparable to the reference standard (2.54 ng/mL). The current approach offers an economical and viable method for the production of biologically active rhIL-2, which can be employed in research and clinical immunotherapy.

Keywords: Recombinant Human Interleukin-2 (Rhil-2), His-Mbp Fusion, Cancer Immunotherapy

1. Introduction

Interleukin-2 (IL-2) is a pioneering immunotherapy for cancer treatment[1].High-dose IL-2 (Aldesleukin) was approved by the FDA for metastatic renal cell carcinoma and malignant melanoma in 1992 and 1998, marking a significant milestone in tumor immunotherapy[2]. IL-2 consists of four α-helices forming a type I cytokine conformation, with three cysteine residues forming two disulfide bridges crucial for its stability and biological activity.Despite its therapeutic potential, rhIL-2 expression in prokaryotic systems often results in inclusion bodies due to its hydrophobic nature[3].

Previous attempts to refold inclusion bodies have been challenging due to IL-2's propensity to aggregate.Alternative expression systems like yeast, insect cells, and CHO cells have been explored but come with their own sets of disadvantages, such as low yields, altered glycosylation patterns, longer production cycles, and high costs[4]. Given these limitations, E. coli remains the preferred host for large-scale rhIL-2 production due to its low cost, rapid protein expression, and compatibility with high-density fermentation[5].

To overcome solubility issues, this study utilized a His-MBP fusion system in the E. coli Origami B (DE3) strain, which contains mutated thioredoxin reductase (trx B) and glutathione reductase (gor) genes to facilitate correct disulfide bond formation and enhance protein solubility.

2. Methods

2.1 Construction of Plasmids

The rhIL-2 protein (UniProt ID: P60568, amino acids 21 to 153) was expressed using a synthetic DNA sequence with a C125S mutation, designed by Tsingke Biotech, Beijing, China, and cloned into the pET-28a vector.For the pET-28a-rhIL-2-MBP-His plasmid, rhIL-2 and MBP fragments were amplified and inserted into the pET-28a vector.The pET-28a-His-Sumo-rhIL-2 plasmid was constructed by amplifying rhIL-2 fragments and subcloning them into the pET-28a His Sumo vector, as indicated in Figure 1.

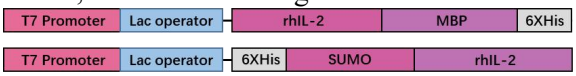

Figure1. Schematics of the Pet-28a-Rhil-2-Mbp-His and Pet-28a-His-Sumo Rhil-2 Vectors

2.2 Expression of Rhil-2 Fusion Protein

The pET-28a-rhIL-2-MBP-His plasmid was

grown in E. coli Origami B (DE3) cells, first growing overnight in 37 °C LB medium with 100 μg/ml ampicillin. The culture was then moved into 2-liter shake flasks of 1000 ml TB medium (100 μg/ml ampicillin) and grown at 30 °C, shaking (200 rpm), to an OD600 of 0.8-1.0. Here, temperature was shifted to 25 °C, and 0.1 mM IPTG was added to induce the expression of the fusion protein overnight. The bacterial cells were harvested by centrifugation (4000 rpm, 30 min, 4 °C) and stored at -20 °C. Expression and expected molecular weight of the pET-28a-rhIL-2-MBP-His protein were confirmed by 15% SDS-PAGE analysis.

The pET-28a-rhIL-2-MBP-His plasmid was grown in E. coli Origami B (DE3) cells overnight in LB medium with ampicillin, then transferred to TB medium and induced with IPTG at 25°C overnight. Cells were harvested by centrifugation and stored at -20°C.Expression was confirmed by 15% SDS-PAGE analysis.

2.3 Purification of rhIL-2 from the Crude Supernatant

Cells were resuspended in a lysis buffer, lysed using a high-pressure homogenizer, and centrifuged.The supernatant was loaded onto a Ni-NTA-Sepharose column, washed, and eluted.Elution fractions were further purified using an MBP column, and the MBP-His tag was cleaved by His-tagged TEV protease.The cleaved fractions were passed through a Hitrap Ni-NTA column, and the flow-through containing purified rhIL-2 was concentrated and stored at -20°C.

2.4 High Performance Size Exclusion Chromatography (HP-SEC)

HP-SEC was performed using a Superdex 200 increase column to ascertain the hydrodynamic volume and elution behavior of rhIL-2.Two mobile phases were used, and samples were loaded using a 500 μl loading loop.Signals were monitored at 280 nm.

2.5 SDS-PAGE Analysis

Samples were prepared with 5× loading buffer, heated, and resolved on 15% SDS-PAGE gels.Gels were stained with Coomassie Brilliant Blue R250, and protein amounts were measured using Glyco BandScan 2.0 software.

2.6 Protein Concentration Assay

Protein concentration was determined using a Bicinchoninic Acid (BCA) assay kit from Sangon Biotech

2.7 Statistical Analysis

Data were analyzed using GraphPad Prism version 9.0, with results presented as mean ± standard deviation (SD).

3. Result

3.1 Plasmid Construction

To construct the pET28a-rhIL-2-MBP-His vector, I initiated the process by amplifying the MBP sequence from our laboratory's plasmid library. A downstream primer was meticulously designed, incorporating a 6×His tag and a termination codon (TAG), to successfully generate the MBP-His fragment.Subsequently, the rhIL-2 sequence was amplified from a commercially synthesized pET28a-rhIL-2 vector using specific primers.Following amplification, the sequences were verified via gel electrophoresis, where target bands were excised and purified to maintain integrity.

The purified MBP-His and rhIL-2 sequences were then mixed in an equimolar ratio (1:1) to serve as templates for assembling the rhIL-2-MBP-His fragment.The fusion PCR, utilizing upstream and downstream primers, successfully yielded the rhIL-2-MBP-His sequence, as depicted in lane 1 of Figure 2. This sequence was then precisely ligated into the pET28a vector using homologous recombination technology.The ligation product was transformed into competent cells, and specific colonies were selected for sequencing to confirm experimental success.Sequencing data confirmed the completeness and accuracy of the sequence.

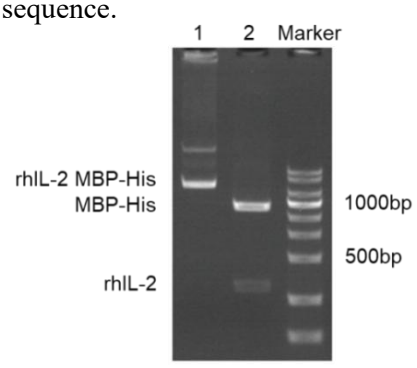

Figure 2. rhIL-2-MBP-His DNA

3.2 Purification of IL-2-MBP-His

For efficient downstream processing, a 6×His

tag was added to the C-terminus of the rhIL-2-MBP-His fusion protein, enabling purification via Ni-NTA affinity chromatography.This method achieved approximately 70% recovery of the recombinant protein, as confirmed by SDS-PAGE densitometry and RP-HPLC (Fig. 3). The MBP-His tag was then cleaved from the fusion protein using TEV protease at a 1:200 mass ratio, resulting in over 95% digestion efficiency, as measured by SDS-PAGE optical density. The digested mixture was passed through a Ni-NTA column in flow-through mode, recovering around 88% of rhIL-2. Impurities, including His-2SUMO, residual rhIL-2-MBP-His, and his-tagged TEVase and MBP-His, bound to the resin. After two-step Ni-NTA purification, the rhIL-2 product reached over 95% purity, verified by 15% SDS-PAGE (Fig. 4). The A280/260 ratio of the purified rhIL-2 was approximately 1.73, with low endotoxin levels (0.05–0.25 EU/μg), indicating minimal contamination risk.

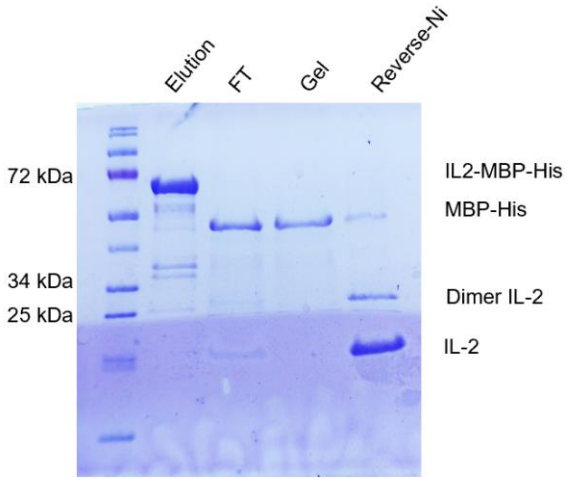

Figure 3.Purification Of Il-2-Mbp-His

3.3 Apparent Molecular Size Analysis

To study the molecular size as observed in purified rhIL-2, HP-SEC analyses were conducted. The results indicated received rhIL-2 eluted at the void volume of column (~7.5ml and 15.0 ml), It indicates that protein I that I obtained contains aggregates and monomers together, Among them, the elution volume of the monomeric protein is slightly lower than that of the HSF protein, the elution volume of the aggregated protein is consistent with that of the BSA protein, indicating that the molecular weight of the IL-2 protein that I obtained is reasonable and its aggregated protein may be a tetramer (Figure 4).

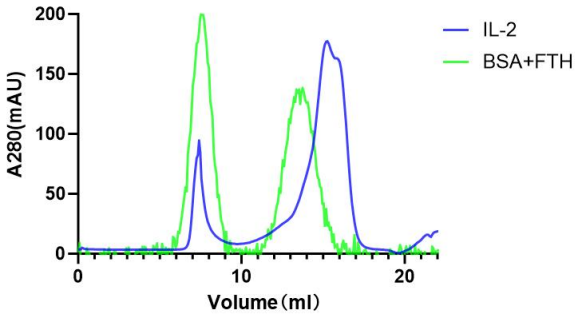

Figure 4. HP-SEC Analysis of the Purified Rhil-2

3.4 Cell Proliferation Bioactivity

Interleukin-2 (IL-2) exerts its biological effects by binding to specific receptors on the surface of target cells, such as the CTLL-2 cell line. The IL-2 receptor (IL-2R) is a multi-subunit complex composed of three distinct chains: IL-2Rα (CD25), IL-2Rβ (CD122), and the common gamma chain (γc, CD132). The interaction between IL-2 and its receptor is highly specific and involves a series of molecular events that ultimately lead to intracellular signaling and cellular responses.

Biological activity of rhIL-2 was determined from its ability to induce cell growth in CTLL-2 cells. Cells were grown without IL-2, and challenged by varying doses of purified rhIL-2 or IL-2 standard. From the data presented, it can be shown that rhIL-2 induced the proliferation of CTLL-2 cells in a dose-related response (Figure5). Further, the dose-response curve for the rhIL-2 was also almost identical with that for the IL-2 reference standard having an equivalent EC50 value of approximately 3.13 ng/ml (compared to 2.54 ng/ml for the standard). These findings confirm that the purified rhIL-2 is active biologically.

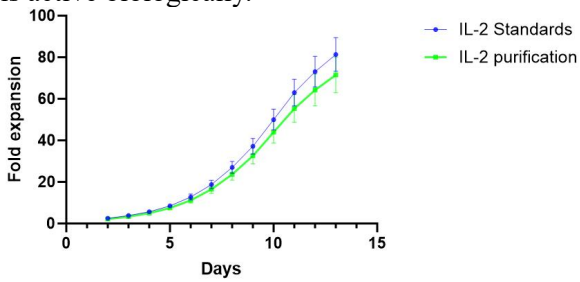

Figure 5. Ctll-2 Cell Proliferation Activity of the Purified Rhil-2

4. Discussion

Production of bioactive recombinant human interleukin-2 (rhIL-2) is critical for cancer immunotherapy. We report an optimized E. coli

protocol that delivers ≥95 % pure, fully active cytokine in three short steps. A His-MBP fusion expressed in the trxB–/gor– Origami B (DE3) strain solubilizes rhIL-2, minimizes inclusion bodies and allows correct disulfide pairing. After IMAC-Ni capture, His-tagged TEV protease removes the tag; a second Ni-NTA passage collects untagged rhIL-2 while discarding uncleaved fusion, protease and endotoxin. The process recovers ~70 % of correctly folded protein; SDS-PAGE and HP-SEC show single-band purity and native monomer–tetramer equilibrium. CTLL-2 proliferation assays give EC50 3.13 ng ml^{-1}, identical to commercial reference. The method is rapid, scalable and immediately applicable for pre-clinical or clinical-grade rhIL-2, offering a robust platform for enhanced cancer immunotherapies.

References

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