The overexpression and purification of soluble interferon alpha in *Escherichia coli*

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Abstract

The soluble expression of human interferon alpha (IFNα) in Escherichia coli will make it simple to be purified. More than ten codons in the vicinity of initiation codon were changed to improve its soluble expression level. It resulted in three-fold improvement of soluble expression level compared to that of original codon-based protein. To make it more soluble, polylysine and ubiquitin tag were fused into the amino terminal of IFNα sequence. To purify IFNα, cleared cell lysates were applied to the cationic ion exchange resin using the buffer of 50mM Tris-HCl (pH7.0) and fractionated in the linear gradient mode of sodium chloride. IFNα containing fractions identified by SDS-PAGE gel running were pooled and treated with deubiquitinating enzyme (DUb) which cleaved carboxy terminal of ubiquitin to render intact IFN α. DUb treated mixture was then applied to the cationic ion exchange resin again, and cleaved IFNα were eluted in the flowthrough fraction due to the overall negative charge of IFNα. Finally, hydrophobic interaction chromatography was followed to further purify IFNα. The purity of recombinant IFNα was confirmed by SDS-PAGE and HPLC. The soluble expression of IFNα and simple purification method can overcome the complexity of purification of insolubly expressed IFNα so far. This method of construction of recombinant protein and purification can be applied to other insoluble proteins and can make industrially applicable.

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