Abstract

Human interferon gamma (IFN-γ) is a cytokine which is critical for innate and adaptive immunity during infection. It is a highly aggregation-prone protein and tends to accumulate in inclusion bodies upon overexpression, especially in heterologous systems. In the present study, we describe the use of an engineered version of IFN-γ which is cloned in E.coli. The expressed protein is produced in both insoluble and soluble forms, with the larger fraction of expressed protein going into inclusion bodies. Our first attempt to obtain functional protein relied on refolding techniques (stepwise dialysis and/or dilution of denaturant from solution) after solubilization of protein from inclusion bodies through the use of denaturant(s). Refolding attempts did not yield properly folded structure, and we turned our efforts towards improving the fraction of soluble expressed protein, through the inclusion of excipients in the growth medium which are likely to enter expressing cells, and found that this led to greater yields of solution protein with lower levels of aggregation-prone behavior. The identity of the recombinant engineered protein was established by mass-spectrometry and further confirmed through western blotting. Further, the engineered protein was characterized to determine its quaternary structural status (oligomerization), folding, chemical and thermal stability using various biophysical techniques. The protein was found to be dimeric. The biological activity of engineered IFN-γ was evaluated by monitoring the activation status of macrophages (HLA-DR expression) and it was found to be an active protein