Recovery of bioactive protein from inclusion bodies using mild solubilisation agent

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Insoluble and **inactive** protein aggregates known as inclusion bodies are the major bottleneck in the recovery of bioactive recombinant proteins from *Escherichia coli*. Strong denaturants such as high concentrations of urea and guanidine hydrochloride offer **good solubility** over a wide range of IBs but result in **poor recovery** of bioactive protein. Recent studies showing the presence of native-like secondary structure in inclusion bodies have led to the development of mild solubilization agents like organic solvents, alkaline pH and low concentration of denaturants. These mild solubilization agents offer 5-6 times better recovery of bioactive protein from inclusion bodies than aforementioned strong denaturants.

In this study, we demonstrated the solubilization potential of trifluoroethanol (TFE), an organic solvent, in nine inclusion body proteins. Different concentrations of TFE with or without low concentration of denaturant were screened to arrive at an optimal ratio. A mixture of 30% TFE with 3M Urea performed the best at solubilizing maximum amounts of protein. Taking human growth hormone (hGH) as a model protein, mode of action of TFE against strong denaturants was investigated using fluorescent spectroscopy and circular dichroism. The results from these techniques suggested the disruption of tertiary structure and stabilization of secondary structure of protein. Furthermore, the cell number based activity assays indicated the presence of fully functional and bioactive protein recovered from TFE solubilized hGH inclusion bodies. We concluded that TFE could be used as a mild solubilization agent to recover maximum amount of bioactive protein from inclusion body proteins.