Solid-phase refolding: Principles and Applications

Chang Woo Suh and Eun Kyu Lee
Bioprocessing Research Laoratory
Department of Chemical Engineering, Hanyang University, Ansan, Korea 425-791

In-vitro renaturation of overexpressed proteins in inclusion bodies has been a bottleneck for large-scale production of recombinant biopharmaceutics. Run-away aggregation of the folding intermediates is considered as the major problem. Various approaches have been studied to circumvent it, and solid-phase (or matrix-assisted) refolding is a viable alternative for a more efficient, high-concentration process. This process allows the proteins to refold as attached to a matrix surface. The attachment forces used were ionic interactions, covalent bonds, hydrophbic interactions, and affinity interactions. Since the intermolecular, protein-protein interactions are avoided, the aggregation can be systematically prevented and thus high-concentration refolding can be achieved.

The solid-phase refolding consists of three steps; itstarts with adsorption of unfolded proteins to a solid matrix in the presence of a denaturant and, if necessary, a reducing agent. The denaturant is washed off the proteins to start refolding. Once the denaturant is completely removed the refolded proteins are revovered by desorption from the matrix.

We have performed several case studies of the solid-phase refolding using various model proteins. They were: (1) a fusion protein of rhGH and GST fragment, (2) a single chain polypeptide of rIFN-alpha, (3) a fusion protein of EGF and angiogenin, and (4) recombinant and modified angiostatin. All of these exploited ionic interactions for adsorption. Other examples were: (5) urokinase that was covalently attached by multi-point amine coupling, and (6) 6x His-tagged enterokinase that was bound to nickel resin by affinity interaction. When the solid-phase refolding principles were applied, all of these proteins demostrated much improved refolding yield and purity compared to the traditional solution-phase refolding.

Furthermore, the solid-phase refolding can be combined with expanded bed adsorpion (EBA) chromatography for EBA-mediated refolding. This process starts with cell homogenate, and can replace the several sequential steps of inclusion body dissolution, cell debris removal, denaturant removal, refolding, and monomer fractionation with an operation inside a single hardware, i.e., EBA column. In this presentation, the background and details involved in the application of the solid-phase refolding and the development of the EBA-mediated refolding process will be discussed.