CGTase Refolding Using Simulated Moving Bed Chromatography

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High expression levels of recombinant protein not only in *Escherichia coli.* but also mammalian often result in the formation of micro-scale particles of aggregated protein, called "inclusion bodies (IBs)". A chromatographic protein refolding technology is receiving more and more attention as a convenient technique to improve refolding yields and to increase the concentration of refolded proteins[1]. However chromatography processes based on a batch operation have typical drawbacks, in-effective solvent utilization, in-sufficient media utilization and high dilution. To address the drawbacks of the batch process, a new continuous refolding method based on a size-exclusion simulated moving bed (SMB) process was developed[2]. Protein refolding based on SMB process can produce refolded protein continuously with high productivity, low consumption of refolding buffer and high efficiency of size exclusion medium. In this study, the extreme thermophilic cyclodextrin glucanotransferase (CGTase) from *Thermoanaerobacter* sp. was successfully refolded from initial high protein concentrations (> 1 g/L). Size exclusion factors and kinetic parameters of CGTase for SMB refolding process were estimated from the best-fit values by comparing the simulation and experimental chromatography results. The SMB operating conditions were obtained from the standing wave design.

References

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