World Academy of Science, Engineering and Technology
International Journal of Biotechnology and Bioengineering
Vol:10, No:02, 2016

Inclusion Body Refolding at High Concentration for Large-Scale Applications

Authors : J. Gabrielczyk, J. Kluitmann, T. Dammeyer, H. J. Jördening

Abstract : High-level expression of proteins in bacteria often causes production of insoluble protein aggregates, called inclusion bodies (IB). They contain mainly one type of protein and offer an easy and efficient way to get purified protein. On the other hand, proteins in IB are normally devoid of function and therefore need a special treatment to become active. Most refolding techniques aim at diluting the solubilizing chaotropic agents. Unfortunately, optimal refolding conditions have to be found empirically for every protein. For large-scale applications, a simple refolding process with high yields and high final enzyme concentrations is still missing. The constructed plasmid pASK-IBA63b containing the sequence of fructosyltransferase (FTF, EC 2.4.1.162) from Bacillus subtilis NCIMB 11871 was transformed into E. coli BL21 (DE3) Rosetta. The bacterium was cultivated in a fed-batch bioreactor. The produced FTF was obtained mainly as IB. For refolding experiments, five different amounts of IBs were solubilized in urea buffer with protein concentration of 0.2-8.5 g/L. Solubilizates were refolded with batch or continuous dialysis. The refolding yield was determined by measuring the protein concentration of the clear supernatant before and after the dialysis. Particle size was measured by dynamic light scattering. We tested the solubilization properties of fructosyltransferase IBs. The particle size measurements revealed that the solubilization of the aggregates is achieved at urea concentration of 5M or higher and confirmed by absorption spectroscopy. All results confirm previous investigations that refolding yields are dependent upon initial protein concentration. In batch dialysis, the yields dropped from 67% to 12% and 72% to 19% for continuous dialysis, in relation to initial concentrations from 0.2 to 8.5 g/L. Often used additives such as sucrose and glycerol had no effect on refolding yields. Buffer screening indicated a significant increase in activity but also temperature stability of FTF with citrate/phosphate buffer. By adding citrate to the dialysis buffer, we were able to increase the refolding yields to 82-47% in batch and 90-74% in the continuous process. Further experiments showed that in general, higher ionic strength of buffers had major impact on refolding yields; doubling the buffer concentration increased the yields up to threefold. Finally, we achieved corresponding high refolding yields by reducing the chamber volume by 75% and the amount of buffer needed. The refolded enzyme had an optimal activity of 12.5±0.3 x104 units/g. However, detailed experiments with native FTF revealed a reaggregation of the molecules and loss in specific activity depending on the enzyme concentration and particle size. For that reason, we actually focus on developing a process of simultaneous enzyme refolding and immobilization. The results of this study show a new approach in finding optimal refolding conditions for inclusion bodies at high concentrations. Straightforward buffer screening and increase of the ionic strength can optimize the refolding yield of the target protein by 400%. Gentle removal of chaotrope with continuous dialysis increases the yields by an additional 65%, independent of the refolding buffer applied. In general time is the crucial parameter for successful refolding of solubilized proteins.

Keywords : dialysis, inclusion body, refolding, solubilization
Conference Title : ICB 2016 : International Conference on Biocatalysis
Conference Location : Barcelona, Spain
Conference Dates : February 15-16, 2016