Purity and concentration of solubilized inclusion bodies in protein refolding

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Solubilized inclusion bodies (IBs) refolding process under low protein purity and high protein concentration conditions always re-aggregate targeted functional protein which is not applicable in nano-biotechnology and molecular biology applications. Enhanced green fluorescent protein (EGFP) IBs was used as the model protein in this study for investigating the effects of protein purity and concentration on the protein refolding process. Three different conditions of solubilized EGFP-IBs were self-refolded at 4°C: solubilized EGFP-IBs with cell debris; solubilized EGFP-IBs after detergent washing; and the purified solubilized EGFP-IBs by using preparative native urea polyacrylamide gel electrophoresis (PAGE). High protein concentration and low protein purity in first and second refolding conditions have resulted re-aggregation of solubilized EGFP-IBs. Large molecular structure of self-refolded EGFP were formed and stuck at the top of stacking and resolving gels during the native PAGE protein analysis. The preparative native urea PAGE has successfully clarified and purified the solubilised EGFP-IBs. The result showed that high purity and low concentration of solubilized EGFP-IBs were able to self-refolded correctly. The structure and biological activity of self-refolded EGFP are preserved and has potential to be used in nano-biotechnologies.

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