MBG FOCUS TALK

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MBG Focus Talks in Molecular Biology

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Like-Charged Surface Facilitates Protein Refolding

Over-expression of recombinant proteins in bacteria such as Escherichia coli (E. coli) often results in the formation of insoluble and inactive inclusion bodies (IBs), so refolding procedure is a critical step in the recovery of functionally active proteins. A big challenge in protein refolding is the aggregation of folding intermediates, which is the main cause of decreased refolding yield. In addition, the contaminants in IBs may also lead to the aggregation of target proteins. So inhibition of the aggregation is the key to the high-performance preparative protein refolding. Moreover, efficient separation and purification of refolded proteins is another important part limiting the large-scale production of recombinant proteins.
We found that charged particles such as ion exchange resins can greatly suppress the aggregation of like-charged folding protein intermediates, leading to the significant increase of protein refolding yield (native protein recovery). The working mechanism of the like-charged surface effect on folding proteins is considered due to the charge repulsion near like-charged surfaces. Namely, the charge repulsion at a solid (or polymer) surface can induce oriented alignment of folding protein molecules, which increases electrostatic repulsion between neighboring folding proteins and leads to the inhibition of protein aggregation. Besides solid particles, the like-charge effect was also confirmed with polyelectrolytes. Detailed research revealed that protein refolding yield increased with increasing ionic capacity and resin concentration. So higher ionic–capacity resin was favorable to offer higher refolding yield at lower added concentrations. Based on the findings, we developed extremely high charge density nanoparticles (4524 μmol/mL) by sequentially modifying silica nanoparticles (SNPs) with poly(ethylenimine) and 2-diethylaminoethyl chloride. Use of the highly charged nanoparticles efficiently facilitated the refolding of like-charged protein at extremely low utilization (e.g., 75% lysozyme refolding at 1 mg/mL with 3.3 μL/mL of the SNPs). Then, we proposed an integrative method of protein refolding and purification by like-charged resin facilitated refolding and metal-chelate affinity adsorption. High efficiency refolding and purification of green fluorescent protein were realized with iminodiacetic acid-grafted SNPs.