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Refolding control of highly disulfide bonded proteins by multi gradient on-column strategy

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Correct formation of disulfide bonds is crucial in the refolding of multi disulfide bonded inclusion body proteins. Covalent aggregation due to wrong cross linking of disulfide bonds leads to a low refolding yield. On-column refolding can be used to refold complex proteins under controlled conditions. This study reports the development of a multi gradient strategy for on-column refolding of high disulfide bonded proteins using size exclusion chromatography. Recombinant tissue plasminogen activator (r-PA, reteplase) with 9 disulfide bonds was used as the protein model. Applying a four-gradient strategy, including decreasing linear gradient of urea concentration and increasing linear gradients of pH, cysteine, and arginine concentration at the same time, resulted in 49.72% activity recovery and 91% mass recovery, which was 4 and 2.5-fold more than conventional on-column refolding with non-gradient and urea-arginine gradient strategies respectively. Successful application of multi gradient strategy in improving refolding yield of reteplase demonstrated controllability of the refolding process using size exclusion chromatography with a rationally designed gradient strategy which can be used in the refolding of other complex proteins too.

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