Development of an E. coli strain for cell-free ADC manufacturing

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Abstract

Recent advances in cell-free protein synthesis have enabled the folding and assembly of full-length antibodies at high titers with extracts from prokaryotic cells. Coupled with the facile engineering of the E. coli translation machinery, E. coli based in vitro protein synthesis reactions have emerged as a leading source of IgG molecules with non-natural amino acids incorporated at specific locations for producing homogeneous antibody drug conjugates. While this has been demonstrated with extract produced in batch fermentation mode, continuous extract fermentation would facilitate supplying material for large-scale manufacturing of protein therapeutics. To accomplish this, the IgG-folding chaperones DsbC and FkpA, and orthogonal tRNA for non-natural amino acid production were integrated onto the chromosome with high strength constitutive promoters. This enabled co-expression of all three factors at a consistently high level in the extract strain for the duration of a five-day continuous fermentation. Cell-free protein synthesis reactions with extract produced from cells grown continuously yielded titers of IgG containing non-natural amino acids above those from extract produced in batch fermentations. In addition, the quality of the synthesized IgGs and the potency of ADC produced with continuously fermented extract were indistinguishable from those produced with batch extract. These experiments demonstrate that continuous fermentation of E. coli to produce extract for cell-free protein synthesis is feasible and helps unlock the potential for cell-free protein synthesis as a platform for biopharmaceutical production.

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