

426r Cell-Free Production of Proteins Requiring Disulfide Bonds

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Cell-free Protein Synthesis (CFPS) is emerging as an important technology for the production of proteins for structural and functional analysis. These post-genomic challenges require a CFPS system that can incorporate post-translational modifications, including the formation of disulfide bonds. The cytoplasm of *E. coli*, and therefore a CFPS reaction using *E. coli* extract, is kept reduced by two parallel pathways mediated by the enzymes glutathione reductase (GR) and thioredoxin reductase (TR).

Proteins requiring disulfide bonds can currently be produced in CFPS by derivatizing the active site cysteines of GR and TR with iodoacetamide, thereby inactivating them. Unfortunately, the iodoacetamide reacts indiscriminately with all reduced sulfhydryl groups in the extract. This leads to a decrease in the protein synthesis yields of these reactions and also inactivates a key enzyme which precludes the use of glucose as an energy source in the CFPS reactions.

We will discuss the development of an *E. coli* strain, and therefore an *E. coli* extract, that allows the formation of disulfide bonds in CFPS reactions without using iodoacetamide. The gene encoding the GR enzyme has been deleted from the chromosome. If both GR and TR are deleted, a peroxiredoxin enzyme mutates to become a disulfide reductase. The gene for TR has therefore not been deleted, but has been modified to include an HA (hemagglutinin) affinity tag. The tagged TR is still active when the cells are grown, but can be removed using an anti-HA column prior to using the extract in a CFPS reaction. We will present cell-free synthesis data for active Tissue Plasminogen Activator (tPA), Urokinase, and Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) requiring 9, 6, and 2 disulfide bonds respectively. CFPS of proteins requiring disulfide bonds using glucose as an economical energy source will also be presented. This technique should be generally applicable to other situations where deleterious enzymes must be removed prior to CFPS, but can not be deleted from the chromosome.