Cancer therapeutic Onconase and lyophilized cell-free protein expression systems

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Introduction: In today's biotechnology world, proteins are engineered and produced to meet many human needs. Cell-free protein synthesis is a particularly useful method of producing important proteins because the reaction environment is not limited to the cytoplasm of living cells. Cell-free synthesis reaction mixtures can also be lyophilized, or freeze-dried, and stored. In this way, protein synthesis reactions can be triggered simply by adding water. Such shelf-stable, on-demand protein synthesis platforms have the potential to impact areas such as biosensing, personalized biotherapeutics and vaccines, biotherapeutic production in remote locations, on-demand biocatalysts, and high-throughput protein screening. We demonstrate the utility of lyophilized cell-free protein expression systems in the production of the anti-cancer/anti-viral protein Onconase.

Materials and Methods: *E. coli* bacteria of the strain BL21.DE3 are grown in LB culture media in the presence of β-D-1-thiogalactopyranoside (IPTG) to trigger the overexpression of T7 RNA polymerase. Bacterial cells are then washed and harvested by centrifugation and homogenized to destroy cell-walls. The cellular lysate is isolated by further centrifugation and then lyophilized, often along with DNA that will code for the protein to be synthesized. For the production of Onconase, DNA plasmids and the energy source phosphoenolpyruvate (PEP) were added along with water to the lyophilized cellular lysate. Cell-free Onconase synthesis reactions were carried out for 3 hours 15 minutes. Anticancer activity of the Onconase was measured with a breast cancer cell line (MCF7) and an MTT (3-[4,5-dimethylthiazol-2yl]-2,5-diphenyltetrazolium bromide) viability assay. Onconase was also expressed in living *E. coli* cells for comparison with that produced in cell-free protein synthesis reactions.

Results and Discussion: Protein synthesis reactions carried out using lyophilized cell extracts produced Onconase at similar yields to standard cell extracts. Additionally, lyophilized extracts were shown to synthesize viable protein after nearly one year of storage. The cancer cell viability assay showed the "Inhibitory Concentration 50" (IC50) for Onconase produced in cell-free protein synthesis reactions, Onconase produced in living *E. Coli* cells, and the cancer therapeutic Doxorubicin to be 110 ± 46 nM, 6480 ± 1440 nM, and 170 ± 70 nM, respectively. Notably, Onconase produced by cell-free protein synthesis reactions showed an even greater anticancer potency than Doxorubicin. Interestingly, Onconase produced in living *E. coli* cells was not as effective at inhibiting cancer cell viability as the Onconase produced through cell-free protein synthesis reactions.

Conclusions: The production of the anti-cancer protein Onconase from lyophilized cell-free protein synthesis reactions demonstrates the utility of these reactions in the on-demand production of important proteins required for applications in biosensing, personalized biotherapeutics and vaccines, biotherapeutic production in remote locations, on-demand biocatalysts, and high-throughput protein screening.

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