Cell-free Protein Synthesis: An alternative tool for discovery and production of proteins

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As the amount of genetic information exponentially increases due to recent advances in genome sequencing, the demand for a faster and more parallel expression system has become greater than ever. It would be nearly impossible to keep pace with the exponentially growing amount of genetic information with current expression technologies. For this reason, cell-free protein synthesis has received renewed attention as an alternative method for high-throughput gene expression. In contrast to in vivo gene expression methods, where protein synthesis is carried out in the context of cell physiology and is surrounded by cell walls and membranes, cell-free protein synthesis provides a completely open system that allows direct access to the reaction conditions. At the same time, most of the cell functions other than protein synthesis need not be maintained in the cell-free system. Thus, it can be optimized with significantly wider latitude than for a living organism. For example, such reaction parameters as pH, redox potential, and ionic strength can be measured directly and changed without concern for harmful effects on the growth and viability of cells. In addition, the products of cell-free protein synthesis are less likely to affect continued productivity. Probably the most promising potential of cell-free synthesis can be found in its suitability for high throughput expression systems. When conducted as a batch reaction, cell-free protein synthesis can be easily expanded into a multiplexed format. However, the poor yield of protein expression in conventional cell-free systems has plagued their practical use and it is an essential prerequisite to improve the productivity of cell-free system for its practical applications. Here we present our recent efforts and achievements to develop a highly efficient cell-free protein

expression system. Through a systematic analysis of the conventional cell-free system, we found that protein synthesis was mainly limited by the supply of ATP due to non-productive degradation of secondary energy source (PEP).

We have devised several methods to utilize the degradation product of PEP for ATP regeneration. In one of those methods, ATP supply was enhanced through the 2-step utilization of energy source, which led to a remarkable increase of protein expression. By combining the new biochemistry of ATP regeneration with CECF(Continuous Exchange Cell-Free protein expression) technology, yield of protein expression reached as high as 6 mg/mL.

Along with several examples of soluble expression of aggregation-prone proteins, we also demonstrate that a complex mammalian protein containing multiple disulfide bonds is successfully expressed in an *E.coli* based cell-free protein synthesis system. Initially disulfide-reducing activities in the cell extract prevented the formation of disulfide bonds. However, a simple pretreatment of the cell extract with iodoacetamide abolished the reducing activity. This extract was still active for protein synthesis even under oxidizing conditions. The use of a glutathione redox buffer coupled with the DsbC disulfide isomerase and pH optimization further enhanced the amount oof active urokinase protease in a simple batch reaction. This result not only demonstrates efficient production of complex proteins, it also emphasizes the control and flexibility offered by the cell-free approach.

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