Secreted Production of Human Erythropoietin Glycoprotein in Non-Lytic Insect *Drosophila* S2 Cell System

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Human proteins often require post-translational modifications for their biological activities. During secretion via endoplasmic reticulum (ER) and Golgi, foreign proteins assemble, fold, and undergo post-translational modifications. Therefore, secretion is a prerequisite to have correct post-translational modifications such as glycosylation. Human erythropoietin (hEPO), with 165 amino acids and a molecular weight of approximately 30 kDa, is a glycoprotein which is a principal growth factor responsible for stimulation of proliferation and differentiation of responsive bone marrow erythroid precursor cells to more mature erythrocytes.

Insect *Drosophila melanogaster* S2 cells were developed as a plasmid-based and therefore, a non-lytic expression system for foreign proteins. We performed successful secreted production of hEPO glycoprotein in S2 cell system.

Transfection is an important step to introduce foreign target DNA into cells and should be properly optimized to obtain maximum production yield. Single factor search (SFS) methodology is still generally used to determine optimal condition in a biological system. Although this method is relatively simple to perform, it has many disadvantages such as not considering interactions between several factors and not covering the entire region of the solution pool. Therefore, we approached this optimization problem statistically with response surface (RSM) and evolutionary operation (EVOP) methodologies, and compared the transfection efficiencies with the traditional SFS method. We employed secreted green fluorescent protein (GFP) as a reporter for determination of optimal transfection condition, and secreted human erythropoietin (hEPO) as a confirming foreign model protein. Consequently, we arrived at the best optimal transient transfection condition through a systematic access in a series of SFS, RSM, and EVOP.

The secreted hEPO yield using optimal transfection condition by EVOP methodology was about 1.8 fold enhanced compared to traditional SFS. A linear relationship between secreted GFP fluorescence intensity and secreted hEPO concentration indicated that facile and noninvasive determination of optimal transfection conditions for expression and secretion of foreign proteins in S2 cell cultures was made possible by simple measurement of GFP fluorescence.

hEPO has been successfully expressed in stable *Drosophila* S2 cells and the molecular weight was about 26 kDa. hEPO was produced in total volume of 250 ml in a 500 ml spinner flask. The cell growth rate and viability rapidly decreased after induction of copper sulfate. Production rate of hEPO quickly increased up to two days after induction and then, slightly increased. We have obtained 5.4 mg/L of hEPO at the maximum point of production rate. The secretion rate was near 97-98%. From this result, the signal sequence, BiP, was proved to work well in producing human EPO in *Drosophila* S2 cells. With a treatment of N-glycosidase F, hEPO without N-glycans had a molecular weight of 18 kDa. This means that the secreted hEPO from S2 cells has a limited glycosylation. From this research, we make sure that *Drosophila* S2 cells produced hEPO in a high yield and BiP can be used in a fused form with hEPO to be secreted out of cells and glycosylation is not fully occurred.

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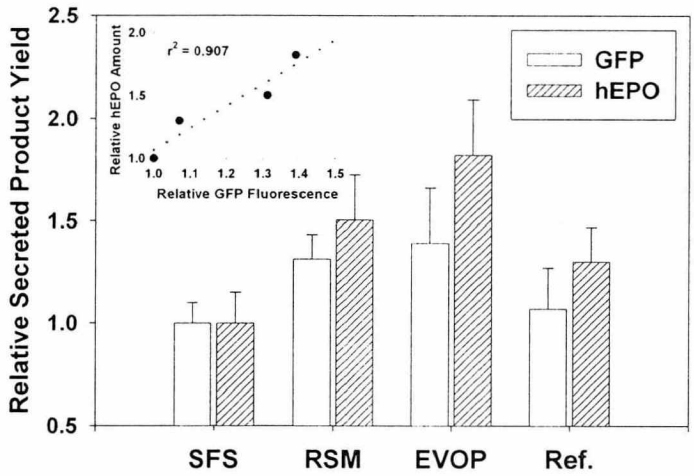

Figure 1. Relative secreted yields of GFP and hEPO under several optimal transient transfection conditions and correlation between relative GFP fluorescence intensity and relative hEPO amount.

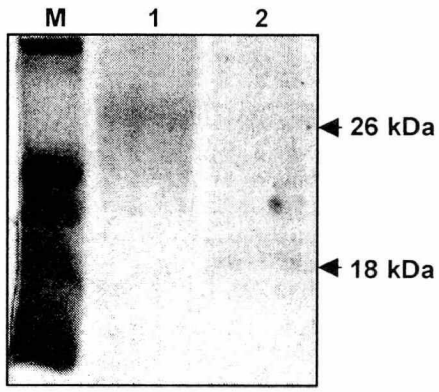

Figure 2. Silver-stained SDS-PAGE analysis of deglycosylated hEPO with the treatment of N-glycanase F. Lane M, protein molecular weight marker; lane 2, hEPO with N-glycans; lane 3, hEPO without N-glycans.