Expression of human erythropoietin (hEPO) in *Drosophila* S2 cells and analysis of *N*-glycosylation

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Abstract

Human proteins often require post-translational modifications, including glycosylation for their biological activities. Insect *Drosophila melanogaster* S2 cell system is a plasmid based, non-lytic expression system and therefore this system has a big advantage for eukaryotic foreign protein production. We performed successful secreted production of human erythropoietin (hEPO) and human transferrin (hTf) in stably transfected S2 cell systems. Transfected S2 cells were produced 18mg/L for hEPO in a 150 mL spinner flask culture and its secretion efficiency near 98"%. Purification of secreted recombinant hEPO and hTf using immobilized metal affinity chromatography (IMAC) yielded 95.5% pure recombinant proteins with a recovery of 32%. According to MALDI-TOF mass spectrometry analysis, purified S2 cell-derived His_6-tagged recombinant hTf had a smaller molecular weight (76.4 kDa) than native apo-hTf (78 kDa). In case of hEPO, purified hEPO from S2 cells has about 23~27kDa of molecular weight and that is also lower than hEPO derived from CHO cells. These data suggest recombinant hEPO and hTf in *Drosophila* S2 cells were incompletely (non-complex) glycosylated and lacked sialic acids on N-glycans. Finally, we analyzed its *N*-glycan patterns through 2-dimensional HPLC with pyridylamino (PA) derivation. The results showed that N-glycans from hEPO has paucimannosidic glycans containing two or three mannose residues with or without core fucose. That means *Drosophila* S2 cell line can be useful as human protein expression system through the development of their *N*-glycosylation pattern to complex type.