Secreted Production and Purification of Human Transferrin in Non-lytic Drosophila S2 Cell System

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Abstract

The human transferrin (hTf) gene, one of the serum glycoproteins related to the transport of Fe^{3+}(1), was fused with hexahistidine affinity ligand and stably transfected into insect *Drosophila melanogaster* S2 cell that was non-lytic plasmid-based system and allowed for secretion of functional human proteins(2). The constructed stable S2 cells secreted almost hTf into the culture medium with 16~18μg/mL yield and 2.7~3.0 μg/mL/day productivity in 150 mL spinner flask culture. We harvested the culture medium at the 3 day post-induction time for the aim of purification of secreted recombinant hTf using an immobilized metal affinity chromatography (IMAC)(3-4). 0.9 mg (about 32% of initial amount) recombinant hTf was purified with high purity (~96%) from 150 mL culture. The purified S2 cell-derived hTf with 76~78 kDa molecular weight was slightly larger than non-glycosylated form (~76 kDa)(5) and smaller than fully-glycosylated native hTf (79~81 kDa) with two simple bi-antennnary N-glycans(6) demonstrating incomplete N-glycosylation in insect *Drosophila* S2 cell system.

References

1. D. M. de Silva, C. C. Askwith, J. Kaplan (1996), Molecular mechanisms of iron uptake in eukaryotes, *Physiol. Rev.* **76**, 31-47.
2. F. Altmann, E. Staudacher, I. B. Wilson, L. Marz (1999), Insect cells as hosts for the expression of recombinant glycoproteins, *Glycoconjugate J.* **16**, 109-123.
3. J. Porath, J. Carlsson, G. Belfrage (1975), Metal chelate affinity chromatography, a new approach to protein fractionation, *Nature* **258**, 598-599.
4. K. L. M. C. Franken, H. S. Hiemstra, K. E. van Meijgaarden, Y. S. J. den Hartigh, T. H. Ottenhoff, J. W. Drijfhout (2000), Purification of his-tagged proteins by immobilized chelate affinity chromatography: The benefits from the use of organic solvent, *Protein Express. Purif.* **18**, 95-99.
5. R. A. Ikeda, B. H. Bowman, F. Yang, L. K. Lokey (1992), Production of human serum transferrin in Escherichia coli, *Gene.* **117**, 265-269.
6. R. A. Ikeda, B. H. Bowman, F. Yang, L. K. Lokey (1992), Production of human serum transferrin in Escherichia coli, *Gene.* **117**, 265-269.