Glycan Analysis of Recombinant Erythropoietin Produced by Glycosyltransferase-Engineered Chinese Hamster Ovary Cells

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Abstract

The attachment of sialic acid residues to glycoproteins can affect important protein properties including biological activity and in vivo circulatory half-life. In order to increase the sialylation of a protein, human α 2,3-ST and β 1,4-GT were engineered into Chinese hamster ovary (CHO) cells which produce recombinant human erythropoietin (EPO). Recombinant human EPO was purified from the culture supernatant by an immunoaffinity chromatography and N-glycans were released from the purified EPO, derivative with 2-aminopyridine, and the relative sialylation of EPO was structually evaluated by DEAE chromatography and 2-D HPLC. When both α 2,3-ST and β 1,4-GT were expressed in CHO cells (GTST15) , more sialylated glycans were produced than those of control (EC1). In detail, relative amounts of di- and tri-sialylated glycans were increased while those of neutral and mono-sialylated glycans were decreased. Specially, tri-sialylated glycans were remarkably increased. In a case of GTST15 cells, the relative portion of tetra-antennary glycans with 3 galactose(Gal) residues was decreased compared to that of control(EC1) cells. Also, tri-antennary glycans with 3 lactosamine (Gal-NeuNAc) units and tetra-antennary glycans with 3 Gal residues and 5 lactosamine units were newly generated in GTST15 cells. The coexpression of the α 2,3-ST and β 1,4-GT, however did not affect to the cell growth and EPO productivity of CHO cells.

References

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