Enhancement of Glycosylation of Therapeutic Protein, Erythropoietin by Expression of Glycosyltransferase in Recombinant CHO Cells

김정희, 임혜림, 최원, 정연태, 손영덕
한국과학기술원 생명과학과
TEL: +82-42-869-2614, FAX: +82-42-869-5614

The attachment of sialic acid residues to glycoproteins can affect important protein properties including biological activity and *in vivo* circulatory half-life. Galactosyltransferase (GT) and sialyltransferase (ST) are responsible for the terminal galactosylation and sialylation, respectively. In order to increase the sialylation of a protein, human α2,3-ST and β1,4-GT were engineered into Chinese hamster ovary (CHO) cells which produce recombinant human erythropoetin (EPO). Recombinant human EPO was purified from the culture supernatant by an immunoaffinity chromatography and *N*-glycans were released from the purified EPO, derivatized with 2-aminopyridine, and the relative sialylation of EPO was structually evaluated by DEAE chromatography and 2-D HPLC(ODS and Amide-80). When both α2,3-ST and β1,4-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. Tri-sialylated glycans from EPO in GTST15 cells were isolated, and micro-structures of glycans were elucidated by 2-D HPLC. Most abundant glycans were tetra-antennary structures. In a case of GTST15 cells, the relative protion 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 galatose residues and 5 lactosamine units were newly generated in GTST15 cells.

References

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