E349 Expression and Characterization of Recombinant Galβ 1,3GalNAcα 2,3-sialyltransferase in Insect Cells

Hyung Gu Kim[1], Su Il Do[2], Young Choon Lee[3] and Jai Myung Yang[1]

[1]Department of Life Science, Sogang University; [2]Molecular Glycobiology Research Unit, Korea Research Institute of Bioscience and Biotechnology; [3]Division of Natural Resources and Life Science, Dong-A University

Galβ 1,3GalNAcα 2,3-sialyltransferase (ST3Gal I) is responsible for the attachment of sialic acid to O-linked glycoproteins and glycolipids. A truncated form of ST3Gal I, lacking 55 amino acids from N-terminus was expressed from recombinant baculovirus-infected insect cells. Prior to recombination, signal peptide of a mouse-derived IgM and protein A for purification was inserted in front of the initiation codon of ST3Gal I gene in frame to make fusion protein. Immunoblot analysis showed that the recombinant ST3Gal I was expressed and secreted into the culture media indicating that IgM signal peptide is effective for secretion of recombinant protein into the media in insect cells. The recombinant ST3Gal I was purified by immunoaffinity column and its biochemical characteristics were analyzed. The recombinant ST3Gal I retains a biological activity that catalyzes the transfer of sialic acid from CMP-NeuAc to the terminal positions of the carbohydrate groups of glycoproteins. These results suggest that massive amount of biologically active form of sialyltransferase could be produce from baculovirus expression system.

E350 Efficiency of Promoters, and 5', 3'-Untranslated Sequences on High Level Expression of Erythropoietin in Mammalian Cells

Jang Hyeon Park[1], Chun Kim[2], Won Bae Kim[1], Young Kook Kim[3], Se Young Lee[4] and Jai Myung Yang[2]

[1]Research Laboratories of Dona-A Pharm. Co., Ltd; [2]Department of Life Science, Sogang University; [3]Korea Research Institute of Bioscience and Biotechnology; [4]Graduate School of Biotechnology, Korea University

Efficiency of viral promoters and 5', 3'-untranslated regions of human erythropoietin (Epo) in directing high level expression in mammalian cells was investigated. In order to analyze the effect of the 5' and 3' untranslated region (UTR) of genomic Epo (gEpo) on the expression level in mammalian cells, 5' UTR deleted, 3' UTR deleted, and 5', 3' UTR deleted gEpos were constructed. These UTR-deleted gEpos were transfected into the COS-7 cells and the amount of transiently expressed Epo was measured. COS-7 cells transfected with the 5', 3' UTR deleted gEpo expressed the highest level, which was 2.6 fold higher than in the COS-7 cells transfected with the wild type gEpo. To investigate the effect of various viral promoters and cell lines on the Epo expression level, the 5', 3' UTR deleted gEpo was cloned next to the SV40 early promoter, CMV early promoter, or SRα promoter. Each of these expression vectors were transfected into the COS-7, BHK-21, and CHO/dhfr- cells, respectively. The COS-7 cells transfected with the vector containing SRα promoter showed the highest expression level (~ 103 IU/ml) at 72 hour of post-transfection. For the development of Epo producing stable cell lines, BHK-21 and CHO/dhfr- cells transfected with the 5', 3' UTR deleted gEpo under the control of SRα promoter were cultured with media containing zeocin. Several clones of zeocin-resistant BHK-21 and CHO/dhfr- cells were cultured in the presence of methotrexate (MTX). A BHK-21 clone selected in the presence of 500 nM MTX expressed and secreted ~ 490 IU/ml Epo into the medium. A CHO/dhfr- clone selected in the presence of 20 nM MTX expressed and secreted ~ 45 IU/ml Epo into the medium. Southern blot analysis done with the probe prepared from the second exon of gEpo indicated that improvement of Epo expression might related with the amplification of gene copy number.