Bacterial expression of recombinant human sialyltransferase, ST6Gal I

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Sialic acid-containing glycoconjugates are involved in a number of biological and pathological events. Many sialyltransferases involved in the synthesis of sialic acid-containing glycoconjugates have been identified. The sialyltransferase ST6Gal I generates α2-6 linkage of sialic acid to non-reducing terminal Galβ1-4GlcNAc residues of oligosaccharides and glycoconjugates, such as glycoproteins and glycolipids.

Influenza viruses infect host cells through specific carbohydrate receptors. Both A and B types of hemagglutinin (HA) recognize specific carbohydrate structures including sialic acid as a receptor. It is thought that interaction of virus hemagglutinin with sialic acid-containing carbohydrates contributes to both interspecies and intraspecies transmission of viruses.

A soluble and active form of recombinant human ST6Gal I was expressed in *Escherichia coli*, which is involved in the biosynthesis of human influenza virus receptor, Neu5Acα2-6Galβ1-R residue. The gene encoding the soluble form of ST6Gal I lacking the membrane and cytosolic regions was introduced into a bacterial expression vector, pCold I, fused in frame with a maltose-binding protein (MBP) tag. A chaperon protein-expressing host bacteria, BL21-Tf16 harboring the resultant plasmid with low-temperature cultivation at 15ºC remarkably contributed to both solubility and MBP-tagging of the recombinant enzyme expressed in bacteria. The recombinant enzyme demonstrated sialic acid transfer activity to both an oligosaccharide and a glycoprotein, asialo-α1-acid glycoprotein, indicating that the enzyme expressed in bacteria is soluble and active. The MBP-tagged enzyme was efficiently purified by amylase-conjugated agarose column chromatography. Soluble recombinant ST6Gal I obtained using our bacterial expression system is a valuable tool to investigate the molecular mechanisms of biological and pathological interactions mediated via carbohydrates.