Elucidation of molecular mechanisms on inhibition of recombinant ST6 by flavonoid derivatives

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Sialoglycoconjugates involved in biological and pathological phenomena. Sialyltransferase ST6Gal I generates α2-6 linkage of sialic acid to non-reducing terminal Galβ1-4GlcNAc residues of glycoproteins and glycolipids. Carbohydrate structures containing Neu5Acα2-6 residues play critical roles in cell-cell recognition and cell-pathogen interaction, such as influenza virus-host cell adhesion. Therefore, chemical compounds that inhibit ST6Gal I has potential for new medicine of influenza infection.

We found some flavonoid derivatives that inhibit sialyltransferase activity, However, inhibitory mechanisms of these derivatives were not been elucidated. Efficient expression system of ST6Gal I is necessary for elucidation of molecular mechanisms. Therefore, we undertook to establish efficient expression system of ST6Gal I. The cDNA encoding a soluble form of ST6Gal I was introduced into a bacterial expression vector, pCold I. The resulted construct generates the enzyme fused in frame with a maltose-binding protein (MBP) tag. This expression vector introduced into *Escherichia coli*, pGro7/BL21 expressing a chaperon, GroES and GroEL. The chaperone protein and low-temperature cultivation during IPTG-induction significantly contributed to improvement of the recombinant enzyme solubility in bacteria. The MBP-tagged enzyme was efficiently purified by a affinity chromatography using amylose-conjugated agarose column. The purified MBP-tagged enzyme was efficiently cleaved by HRV3C protease. Protease treatment remarkably enhanced sialic acid transfer activity to asialo-α1 acid glycoprotein.

In conclusion, we established efficient expression system of soluble ST6Gal I in *E.coli*. This system will be used for elucidation of molecular mechanism on ST6Gal I inhibition by flavonoid derivatives. That would lead to development of new medicines derived from food materials.