Probing the Trans-Sialidase Activity of the Neuraminidase Derived from Human Influenza A (H3N2) Virus

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ABSTRACT

Sialo-oligosaccharides are found on the distal ends of glycans present on mammalian cell surfaces. They play major roles in cell growth, function, and are determinants of many disease states. Due to the important physiological role of glycoconjugates, there has been increased focus towards the development of drugs and vaccines that are carbohydrate based. Sialo-oligosaccharides can be synthesized through chemical means or by use of enzymes. Enzymes are preferred in the synthesis process as they are cheaper, faster and capable of catalyzing the synthesis of molecules with the correct specificity. These being the case enzymes with activities capable of synthesizing glycoconjugates are being sought. Like other glycosidases, neuraminidases, also referred to as sialidases, hydrolyse glycosidic linkages. Glycosidases are also known to catalyze the synthesis of oligosaccharides through trans-glycosylation, but the trans-sialidation potential of Influenza neuraminidases has not been investigated. This aim of this study sought was to determine whether the influenza A H3N2 neuraminidase has trans-sialidase activity. The influenza A H3N2 virus was amplified by inoculating in Madin Darby canine kidney (MDCK) cell line. Total viral RNA was extracted and reverse transcribed. The cDNA was used in the amplification of neuraminidase Type 2 (NA2) followed by cloning, sequencing and expression of the neuraminidase (NA2). The recombinant enzyme was used in desialylation of guinea pig red blood cells to establish the presence of sialidase activity. The trans-sialidase activity was assayed using sialic acid from RBC as donor and PNP β-D galactose as the acceptor molecule. Products of trans-glycosylation reaction were analysed by Thin Layer Chromatography (TLC) plate and by capillary electrophoresis.

Hemagglutination Inhibition of guinea pig RBC occurred after treatment with the recombinant influenza A type 2 neuraminidase. This showed that desialylation had taken place and hence the

sialidase activity of the recombinant neuraminidase was present. The resolution of transglycosylation reaction on TLC and by capillary electrophoresis did not reveal any products. This observation suggests that there was no transfer of sialic acid residues from donor RBC to acceptor molecule PNP-Gal. These findings suggest that the trans-sialidase activity of neuraminidase derived from influenza A/Nairobi/2041/2006(H3N2) is not significant. The search for enzymes that can be used for synthesis sialo-oligosaccharides continues. In future studies on the neuraminidase (NA2) trans-sialidase activity, the purification method should be improved by incorporating a Histag on the expression vector. The analysis of the trans-glycosylation products should be carried out using approaches such as mass spectroscopy as it is highly sensitive and able to detect compounds in trace quantities that are not detectable by Thin Layer Chromatography and Capillary Electrophoresis. Since the use of chemicals in the synthesis of carbohydrates remains expensive and full of challenges in reference to regiospecificity, it would be worthwhile to carryout molecular evolution to transform A/Nairobi/2041/2006(H3N2) neuraminidase to a trans-sialidase.