CONTROLLING SIALYLATION LEVELS WITH A NOVEL α-2,6-SIALYLATRANSFERASE

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α2,6 sialylation enhances the immunomodulatory role of IgG-type monoclonal antibodies (mAbs), ultimately increasing therapeutic efficacy and reducing intrinsic side effects compared to their asialylated counterparts [1]. Current mAb manufacturing platform is unable to incorporate sialic acid at the α2,6 positions, but this can be achieved through *in-vitro* glycoengineering (IVGE). Prior to the α2,6-sialylation, enzymatic IVGE requires a galactosylation step using human ß1,4-galactosyltransferase and UDP-galactose. For α2,6-sialylation, human α2,6-sialyltransferase (α2,6SiaT) is preferred due to its high efficiency in generating di-sialylated (S2) glycoforms and low CMP-Neu5Ac hydrolytic and sialidase activities [2]. However, its limited availability and its high costs hinder its commercial applicability. In contrast, bacterial α2,6-sialyltransferases are a more economical and readily available alternative, but exhibit high CMP-Neu5Ac hydrolytic and sialidase activities that are difficult to counteract with alkaline phosphatase or other additives to achieve high levels of sialylation [2-4]. Another significant hurdle is the cost of CMP-Neu5Ac.
This study introduces a novel bacterial non-hydrolytic α2,6-sialyltransferase that lacks hydrolytic activity towards CMP-Neu5Ac, thus, eliminating the need for alkaline phosphatase. Coupled with low-cost CMP-Neu5Ac, and following a preliminary galactosylation step, Rituximab from two sources underwent α2,6-sialylation in the absence of alkaline phosphatase. Approximately, 35% S2 and 40% mono-sialylated (S1) glycoforms were achieved for Rituximab produced in CHO cells, while around 90% S2 and 5% S1 were achieved for Rituximab produced in High Five™ insect cells. Overall, the presented approach constitutes a feasible and practical alternative for the *in-vitro* enzymatic α2,6 sialylation of monoclonal antibodies.

References:

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