Neisseria meningitidis is one of the causes of bacterial meningitis in the world. Most cases of the disease affect children under the age of 2 and cause high rate of mortality (up to 50%). There are 12 different N. meningitidis serogroups and six of them (A, B, C, Y, W, and X) can cause bacterial meningitis. Each serogroup of Neisseria meningitidis has different polysaccharides in the capsule. Disease-causing serogroups A, B, C, and X contain polysaccharides that are homopolymers, while those of serogroups W and Y are heteropolymers. N. meningitidis serogroup W capsular polysaccharide is a heteropolymer of galactose and sialic acid (Fig. 1). This heteropolymer is synthesized by the N. meningitidis serogroup W capsule polysaccharide which uses CMP-Sialic acid, UDP-Galactose, and DTT (100µM each) as nucleotide donor sugars. The serogroup W enzyme is understudied in the literature, but some biochemical data is known. According to Romanow et al. (2013), the enzyme has three different domains. The N-terminal domain is the hexosyltransferase domain responsible for transferring galactose, the C-terminal domain is the sialyltransferase domain which is responsible for transferring sialic acid. The intervening sequence separates the hexosyltransferase and sialyltransferase domains (Romanow et al., 2014). However, the catalytic domain of the enzyme is still unknown. We aim to determine these parameters and optimize enzyme activity for glycoconjugate vaccine development. One goal is to control the activity of the enzyme to control the size of the polysaccharide.

**MATERIALS AND METHODS**

**W135 Polysialyltransferase Growth, Expression, and Purification**

Neisseria meningitidis was grown and expressed in E. coli KRX cells according to a published procedure (Shey et al.). Nickel affinity chromatography was performed according to a published procedure. Purified enzyme was characterized by Bradford reagent and SDS-PAGE electrophoresis.

**CMP-Glo Bioluminescence Assay**

Biotransferase assays were performed with different amounts of purified serogroup W enzyme (0-150, 125, 250, 500, 750, 1000, and 1250 ng for reaction). CMP-Sialic acid (100µM each), Hydrolyzed serogroup W Acceptor (0.4mg/ml), in buffer (50 mM Tris, 20 mM MgCl2, 2mM DTT, pH 8.0). Reactions were run for 1 hr at room temperature (RT). Reactions were quenched after 1 hr by adding CMP-Glo Nucleotide Detection Reagent (NDR). High luminescence was seen in the presence of all components. Km and Vmax values for acceptor in the literature.

**UDP-Glo Assay Using Sialic Acid Trimer (DP3) as an Acceptor**

In this work we use CMP and UDP-Glo Bioluminescence assays to determine kinetics. Free CMP and UDP which are produced by enzyme activity and changed to ATP by adding Nucleotide Detection Reagent (NDR). The synthesized ATP is converted to light and luminescence is measured.

**RESULTS**

**CMP-Glo Assay Using W-Hydrolyzed Sugar as an Acceptor**

Km and Vmax values for W-Hydrolyzed Sugar acceptor in the reaction are 305.2 µg/mL and 0.0089 µM/min respectively.

**UDP-Glo Assay Using Sialic Acid Trimer (DP3) as an Acceptor**

The optimum amount of serogroup W capsule polymerase enzyme that can be used in the UDP-Glo assay is 1250 ng. Maximum amount of enzyme, shows maximum activity.

**CONCLUSIONS**

All kinetics assays in this research were done in the presence of homogenous acceptor (DP3) by UDP-Glo Bioluminescence assay. We are going to make new modified acceptor UDP-Galactose to determine Km and Vmax for this acceptor and CMP-Sialic acid.

**FUTURE WORK**

By using modified CMP-sialic acid (CMP-Sia-Daz), we are going to do photo crosslinking to find critical amino acids to control the activity of the enzyme and therefore control the size of the polysaccharide.

**REFERENCES**


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