Chemoenzymatic Strategies to Investigate Activity of a Bifunctional *Neisseria meningitidis* Capsule Polymerase

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***Neisseria meningitidis* is one leading cause of bacterial meningitis**

- The Gram-negative bacteria is surrounded by a coat known as the capsule
- The capsule is rich with long polymers of carbohydrates (polysaccharides)
- This capsule protects bacteria against killing by the human host
- Serogroups A, B, C, X, W and Y are responsible for many of cases of meningococcal meningitis.
- Meningitis belt of sub-Saharan Africa – estimated to be up to 1000 cases per 100,000 vs. up to 3 cases per 100,000 elsewhere (cdc.gov)

Capsular Polysaccharides from *N. meningitidis*

- 6 of 13 serogroups of *Neisseria meningitidis* cause most cases: A, B, C, W, X, and Y
- Serogroups defined by capsular polysaccharides

McCarthy et al. *Vaccines* **2018** 6, 12

R and Ac = acetylation

Glycoconjugate Vaccines

PS hydrolyzed and chemically derivatized at hydroxyl or carboxyl groups

+

Chemically derivatized at amino or carboxylate groups of amino acids in carrier protein

- Random, multipoint attachment
- Heterogeneous mixtures
- Relationship between vaccine structure vs. immune response is less clear

Goal: Well-Defined Glycoconjugate Vaccines

Controllable synthesis of sugars with one site for conjugation

+

Modification of carrier protein at known sites

McCarthy et al. *Glycocon. J.* **2013** 30, 857
Peterson et al. *J. Bacteriol.* **2011** 193, 1576
Mosley et al. *Biocat. Biotrans.* **2010** 28, 41

Capsular polysaccharides define *N. meningitidis* serogroups

R and Ac = acetylation

- Capsules of serogroups A, B, C, and X each contain polysaccharide chains comprised of one carbohydrate

- Capsules of serogroups W and Y each contain polysaccharides comprised of two carbohydrates

***N. meningitidis* serogroup W = Heteropolymer of galactose and sialic acid**

***N. meningitidis* serogroup Y = Heteropolymer of glucose and sialic acid**

The *N. meningitidis* serogroup W Capsule Polymerase

Romanow et al., *J Biol Chem* **2014** 289, 33945

Romanow et al., *J Biol Chem* **2013** 288, 11719

Claus et al., *Mol Microbio* **2009** 71, 968

The *N. meningitidis* serogroup W Capsule Polymerase

Romanow et al., *J Biol Chem* **2014** 289, 33945

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Research Questions

- Can we use the recombinant form of the enzyme for **controlled polysaccharide synthesis**?
- Can the enzyme use alternate substrates to make new potentially useful polysaccharide structures?

Research Questions

- Can we use the recombinant form of the enzyme for **controlled polysaccharide synthesis?**
 - Targeted site-directed mutagenesis: chemoenzymatic synthesis of compounds to help determine amino acid sites to target
 - Chemoenzymatic synthesis of competitive inhibitors/chain-termination agents

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Chemoenzymatic Synthesis of a Photocrosslinking Nucleotide Donor Sugar

- Photocrosslinking of *N. meningitidis* serogroup W potential active site residues using ManN**DAz** (generous gift of Dr. Jennifer Kohler, UT Southwestern Medical Center and Dr. Matthew Pratt, USC)

2-Acetyl-*N*-4,4-azo-pentamido-2-deoxy- α,β -D-mannopyranose (ManNDAz)

One Pot Chemoenzymatic Synthesis of CMP-SiaDAz

Monitor production of CMP-Sia or CMP-SiaDAz products by Anion Exchange HPLC

One Pot Chemoenzymatic Synthesis of CMP-Sia

- * Represents peak verified to be CMP-Sia
- Observe decrease in peak around retention time ~ 16 min

One Pot Chemoenzymatic synthesis of CMP-SiaDAz

- Consistently observe increase in peak around retention time ~ 16 min
- Working to optimize reaction conditions and optimal separation techniques

One Pot Chemoenzymatic synthesis of CMP-SiaDAz

- Determine sites of crosslinking by MS (collaboration with Dr. Lukasz Joachimiak, UT-Southwestern)
- Evaluate impact on activity after site-directed mutagenesis

An Easy, Sensitive Assay is Key to Evaluate Wild Type vs. Mutant Activity

This enzyme lacks a facile assay for unlabeled acceptors. We have investigated assays based on:

- Fluorescence
- Absorbance
- **Bioluminescence**

Bioluminescence Assays

Nacalai USA

Acceptor: Sialic acid trimer (DP3)

Commercially available kits from Promega:

UDP-Glo is the most tractable vs. CMP-Glo with this acceptor

Converted to light

UDP-Glo Assay: Sialic Acid Trimer is a More Suitable Acceptor

UDP-Glo assay

Galactosyltransferase Activity: High affinity for UDP-Galactose

Vmax	0.009187
Km	39.35

R square	0.954
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Sialyltransferase Activity:

Enzymatic synthesis of galactose terminal acceptors

<u>Varied component</u>	<u>Constant components</u>	<u>Acceptor used</u>	<u>Activity</u>
UDP-Gal	Acceptor, CMP-Sia	DP3	Galactosyltransferase
CMP-Sia	Acceptor, UDP-Gal	Gal-DP3	Sialyltransferase
Acceptor	CMP-Sia, UDP-Gal	DP3	Galactosyltransferase
Acceptor	CMP-Sia, UDP-Gal	Gal-DP3	Sialyltransferase

Enzymatic addition of galactose by the W enzyme

-W +W

Recently

- Work from Xi Chen (UCSD): *ACS Catalysis* **2020** 10 (4), 2791-2798
- Chemoenzymatic synthesis of chromophore labelled acceptor
- Quantitation of products by HILIC-LC
- Their kinetic studies suggest increasing chain length of acceptor → better substrate
- Currently evaluating kinetics of increased chain length acceptors in our assay

Research Questions

- Can we use the recombinant form of the enzyme for **controlled polysaccharide synthesis?**
 - Chemoenzymatic synthesis of competitive inhibitors/chain-termination agents

Chemoenzymatic synthesis of CMP-4-Acetyl-Sia

N. meningitidis CSS

CMP-4-Acetyl-Sia

*CMP-4-Acetyl-Sia should act as
a chain termination agent and
decrease chain length*

An Easy, Sensitive Activity Assay is Key

We have investigated assays based on:

- **Fluorescence: DMB-labelled serogroup W oligosaccharide acceptor**
- Absorbance
- Bioluminescence

Serogroup W Reaction with CMP-Sia vs. CMP-4-Acetyl-Sia

*New peaks with enzyme
indicate product formation*

Serogroup W Reaction with CMP-Sia vs. CMP-4-Acetyl-Sia

CMP-4-Ac-Sia

*Decreased product formation with
CMP-4-Acetyl-Sia*

*Still some product formed potentially
from 2→6 linkage to Gal*

Research Questions

- Can we use the recombinant form of the enzyme for **controlled polysaccharide synthesis?**
 - Targeted site-directed mutagenesis: chemoenzymatic synthesis of compounds to help determine amino acid sites to target
 - Chemoenzymatic synthesis of competitive inhibitors/chain-termination agents

Future Work

- Kinetic studies: Finish kinetic studies
- Photo-crosslinking: Continue optimizing CMP-SiaDAz reaction and perform crosslinking studies, MS/MS to identify sites of modification, site-directed mutagenesis and assess affects on activity
- Competitive inhibitors: Continue work with CMP-4-Ac-Sia and other potential modifications to Sia
- Glycoconjugates: Conjugate well-defined carbohydrates to carrier proteins

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