Examining the glycosylation pathway in *Helicobacter Pylori* using mass spectrometry
Catherine Liu, 2019

The pathogenic bacterium *Helicobacter pylori* (*Hp)* is responsible for duodenal ulcers as well as stomach cancer, the second leading cause of cancer death worldwide (1). However, there is growing concern for *Hp's* ability to resist current antibiotic treatments (2). In addition, current antibiotic treatments are non-specific for pathogens, and kill the advantageous stomach bacteria (2). Thus, there's a need to develop new treatments that will specifically target *Hp.*

The glycoproteins in *Hp* represent an intriguing target for therapeutics, since their glycan structures are distinctive from their eukaryotic counterparts (3). They also often play an important role in pathogenesis. Pathogenic bacteria that cannot synthesize glycoproteins have shown a range of defects, including reduced adhesion to host cells, defective biofilm formation, and disrupted flagellin formation, leading to an immotile pathogen that cannot colonize the host (4, 5). Thus, understanding the glycosylation pathway and the enzymes involved can help further elucidate how to target these glycoproteins.

Previous work in the Dube lab has identified 13 *Hp* glycosyltransferase genes required for glycoprotein biosynthesis. To achieve this goal, the Dube lab developed 13 *Hp* G27 mutant strains containing insertions in these glycosyltransferase-encoding genes. These thirteen mutants displayed reduced glycoprotein production compared to wild type, and intriguingly, there was notable similarity of glycoprotein labeling across all mutant strains (6). These results indicated a common biosynthetic pathway, and indicated that these 13 *Hp* genes were involved in this pathway. However, the mechanisms of the glycosylation pathway and how these enzymes work together is still unknown. Understanding the mechanisms of this pathway will shed light on understanding how glycan structure relates to its function in pathogenesis, as well as enable the development of novel glycosylation-based drug targets.

This summer, I sought to understand the glycosylation pathway by using mass spectrometry as an analytical tool. Mass spectrometry can fragment the bonds within a chemical species, and measure the masses of the resulting fragments. Since glycans tend to fragment along the bonds that connect their monosaccharide building blocks, and these units have characteristic masses, we can use mass spectrometry data to potentially build up the entire glycan structure. Using mass spectrometry, I hope to examine the glycan structures on the 13 *Hp* G27 mutant strains as well as on wild-type *Hp,* and I hypothesize the mutant strains will contain truncated glycans compared to wild type. The extent of glycan complexity will reveal the role of each glycosyltransferase enzyme, and determine its relative placement in the enzymatic pathway. Thus, using mass spectrometry analysis of glycan structure, I hope to piece together the full model of glycan assembly.

I began by establishing a method to examine glycan structure in *Hp* using mass spectrometry. My approach included lysing *Hp* cells, digesting the whole cell lysate using the protease trypsin, and analyzing the resulting glycopeptides using liquid chromatography mass spectrometry (LCMS). Applying this approach to wild-type *Hp,* I detected peptides but I was unable to detect glycans in the LCMS data, most likely because the peptides signals were much stronger than the glycan signals. Thus, enrichment of glycopeptides within my samples was necessary.

Next, I tested two methods of glycopeptide enrichment that had been well-established in eukaryotic systems but were untried in bacterial systems: hydrophilic interaction liquid chromatography (HILIC) and strong anion exchange chromatography (SAX) (7). Glycopeptides come in two varieties; they are either N-linked (attached to asparagine amino acid residues) or O-linked (attached to serine or threonine residues). I first tested HILIC using a series of well-characterized eukaryotic glycoproteins as well as wild-type *Hp* samples, and I found that HILIC effectively retained elaborated N-linked glycopeptides, but were ineffective for enriching O-linked glycopeptide controls as well as wild-type *Hp* glycopeptides. As an alternative option, I tested the SAX method using the same set of controls, and my results were inconclusive, as no glycopeptides were detected in both non-enriched and SAX-enriched samples across all controls. Further experiments are necessary to test the efficacy of the SAX method for enriching O-linked glycopeptides. For my future direction, I hope to establish an effective method of enriching glycopeptides in *Hp* and examining their glycan structures using mass spectrometry. Ultimately, I hope to use this approach to better understand the glycosylation pathway in *Hp.*

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