Structural Identification of *Helicobacter pylori*'s Glycoproteins

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Helicobacter pylori (*Hp*) is a Gram-negative, pathogenic bacteria that colonizes the gastric tract of over 50% of humans and can cause ulcers and gastric cancer (1). As antibiotic resistant strains become more common, new methods of treatment are needed. One characteristic of *Hp* that could be used as a potential target for therapeutics is the link between its ability to glycosylate proteins and its pathogenesis. Glycosylation is crucial for proper flagella formation, motility, and colonization, so without glycosylation, *Hp* is unable to cause infection (2). More knowledge about these glycosylated proteins, such as the sugar structures, could lead to the development of novel therapeutics. This characteristic of *Hp* is especially interesting as a target because bacterial glycans contain unique structures that do not appear in human cells (3). Metabolic oligosaccharide engineering, a process that replaces natural sugars with slightly modified monosaccharaides through the bacteria's natural metabolic process, facilitates labeling and detecting *Hp*'s glycosylated proteins (4). For this project, these modified monosaccharaides have an azide chemical tag that can be targeted to react with phosphine TAGs, which aid in purification and identification. Previous results using liquid chromatography mass spectrometry (LC-MS), a method of analysis that provides structural information about compounds, have produced spectra that do not allow for sugar analysis because intense TAG peaks overpower small sugar peaks (4). This result may occur because product ions formed from the LC-MS need to be charged to be detected, but no basic sites are present on the sugar while several basic sites are present in the TAG.

This summer, my work included synthesizing and analyzing different TAGs to see if decreasing the number of basic sites on the TAG increases the intensity of the sugar peaks. While the previous results that provided little information about the sugar structure had TAGs that produced charge states between +2 and +5, where higher charge states indicate a greater number of basic sites, the synthesized TAGs of interest this summer produced charge states that were either +1 or +2. Standards of the new TAGs with the glycan attached have not yet been analyzed, but LC-MS spectra of the TAGs have been analyzed for characteristic peaks produced from losses from the molecule such as water, methanol, or amino acid, to enable easier analysis of the Glycan-TAG spectra. The next steps for this project include characterizing Glycan-TAG spectra to determine the relationship between basic sites and sugar peak intensity and using energy resolved mass spectrometry to clarify what collision energy provides the best structural information about the sugar.

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