Addition of Basic Sites to the Glycans of *Helicobacter pylori* to Increase MS/MS Peak Abundance

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The bacteria *Helicobacter pylori* is the leading cause of duodenal ulcers, gastritis, and gastric cancer worldwide.¹ Not only does the current treatment for an *H. pylori* infection disrupt the populations of beneficial bacteria within patients for up to four years, but it is also becoming increasingly ineffective as *H. pylori* develops resistance to these antibiotics.² Therefore, there is an extreme demand for new methods of treatment for *H. pylori* infections.

The sugars, or glycans, present on the surface of *H. pylori* are directly linked to this organism’s ability to be a pathogen. For example, the presence of the bacterial sugar pseudaminic acid on the proteins of the *H. pylori* flagellin is essential to the bacteria’s ability to form functional flagella and inhabit the host’s stomach.³ The exclusive presence of this glycan on the flagellin protein suggests that its structure is vital to *H. pylori*’s ability to cause disease. However, the structures of *H. pylori*’s glycans remain largely unknown. Ultimately we are interested in determining glycan structures in order to aid the development of novel therapeutics to eradicate *H. pylori*.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is a powerful tool for structural identification. This instrument separates various components of a sample solution, ionizes the molecules present by attaching protons to any basic sites, and then dissociates the ions formed into fragments. These fragments produce measurable signals based on their mass and charge that can be used to determine the structure of the ion from which they came. During previous attempts, Scott Longwell ’12 and coworkers found it challenging to gather structural information about *H. pylori*’s glycans using LC-MS/MS analysis as the fragment peaks of the phosphine-based probes used to isolate the glycans, generically called Phos-TAGs, dominated the MS/MS spectra produced.⁴ Weak signals from the glycan peaks ultimately hinder the accurate determination of the unknown sugar structures.

We hypothesized that the low signal intensity of the glycan peaks is due to a lack of basic sites on the glycans where a proton could be added. As a result, when the glycan-Phos-TAG ion fragments, the glycan is lost as a neutral molecule that the instrument cannot detect. Reductive amination offers a means by which a basic site can be added to the sugar as the aldehyde present in the open ring form of the glycan end of the molecule reacts with an amine that contains an additional basic site. By improving the sugar’s ability to retain a positive charge, we hoped to increase the sensitivity of the instrument to the glycan fragments, therefore leading to more informative peaks and more complete glycan characterization.

My goals for the summer were to successfully perform a reductive animation of the synthetic glycan-Phos-TAG standard, GlcNAz-Phos-FLAG, and to find an amine that would produce glycan peaks in the MS/MS spectra that were more abundant than the Phos-FLAG peaks. Over the course of the summer I was able to reductively animate GlcNAz-Phos-FLAG using three amines: 2-aminobenzamide, ethylenediamine, and 2-2‘(ethylenedioxy)bis(ethylamine). Of these amines, 2-2‘(ethylenedioxy)bis(ethylamine) produced glycan fragment peaks that were more abundant than the Phos-FLAG peaks; however, there was also evidence that this amine was undergoing a second reductive animation with unreacted GlcNAz, thereby creating undesired side products. As a result, we have decided to proceed using the monofunctional amine N,N-dimethyl-p-phenylenediamine.

Ultimately, if reductive amination successfully increases the glycan signal strength, it will be possible to use this reaction to determine the structure of sugars that are found in samples from *H. pylori*. An understanding of *H. pylori*’s glycan structures will aid the development of therapeutics that can alter the sugars or prevent their addition onto the surface of the bacteria, thereby rendering *H. pylori* innocuous.
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References