Profiling the Subcellular Localization of *Helicobacter pylori*’s Glycoproteins

Scott Longwell, 2012

The Gram-negative bacterium *Helicobacter pylori* is a growing problem. More than half the human population harbors the microbe within the mucosal lining of their stomachs, making it one of world’s most common infections. While roughly 80% of these individuals are asymptomatic, the remaining 20% are afflicted with severe gastritis and show greatly elevated rates of ulcer formation and gastric cancer. The current option for eradicating *H. pylori*—a “triple-therapy” cocktail of high-strength antibiotics and proton pump inhibitors—generally works well. However, *H. pylori* has proven to be a rugged, quickly mutating species, and many strains have become resistant to standard eradication procedures. Clearly, the development of replacement treatments is needed and will require identification of new molecular targets within *H. pylori*.

Glycoproteins constitute one such class of potential targets. These are proteins that have undergone a post-translational modification to attach carbohydrates—glycans—to their structure. Often, this modification is required for the proper folding, localization, or general function of a glycoprotein. It was previously believed that the bacterial domain was devoid of the molecular machinery required to add glycans to proteins. However, a wide range of studies has now demonstrated that some bacteria, including *H. pylori*, have a significant glycoprotein library. For instance, it was recently discovered that *H. pylori*’s flagellar proteins must be glycosylated (have glycans added) in order for the microbe to be motile and virulent.

In the quest to inventory *H. pylori*’s glycoproteins, a technique termed metabolic oligosaccharide engineering (MOE) has proven to be particularly effective. At its core, this method utilizes peracetylated N-azidoacetylglucosamine (Ac₄GlcNAz), an unnatural glycan building block modified with an azide. The glycosylation machinery responsible for constructing *H. pylori*’s glycan cannot recognize the difference between this sugar and its natural counterpart, N-acetylglucosamine (GlcNAc). As a consequence, *H. pylori*’s own biosynthetic pathways will incorporate Ac₄GlcNAz into many of its glycoproteins upon incubation with the unnatural sugar. At this point the azide group, which is biologically inert, acts as a handle for *H. pylori*’s glycoproteins: it can be selectively reacted to link with a phosphine tag (Phos-FLAG-His₆) via the Staudinger ligation. Any glycoprotein bearing this tag can then be isolated with affinity chromatography and characterized with immunoblotting and mass spectrometry (MS). Using MOE, previous work by Maria Koenigs ’09 and Kanokwan Champasa ’11 has already uncovered far more glycoproteins than were originally thought to exist in *H. pylori*, some of which of been characterized as known virulence factors.

Recently, the Dube lab has investigated the question of what glycoproteins are present in *H. pylori*. My own research continues to address this question, as well as the refining question of where these glycoproteins are located within the cellular structure of *H. pylori*. In general, the proteins of a Gram-negative bacterium may be classified as cytoplasmic (within the inner membrane), periplasmic (between the inner and outer membrane), or secreted (outside of the cell). Additionally, proteins may associate directly with either the inner or outer membranes in any number of orientations: they may be attached to the interior or exterior of a membrane, or span part or all of the membrane. As a subclass of proteins, glycoproteins may localize to any of these subcellular regions. My work thus far has been to determine the distribution of glycoproteins across these regions by utilizing selective membrane lysis and differential centrifugation to fractionate *H. pylori* cells and MOE to visualize the glycoprotein profiles of each fraction. Access to this information will yield insight into the role of glycoproteins in *H. pylori*’s physiology and may reveal new drug targets.

To date, subcellular fractions have been prepared from *H. pylori* cultures treated with Ac₄GlcNAz on two occasions. These fractions were reacted with Phos-FLAG-His₆ (so as to tag azide-labeled glycoproteins). An anti-FLAG immunoblot of both sets of cellular fractions revealed azide-labeled glycoproteins in the secreted, periplasmic, inner-membrane, and cytoplasmic fractions, suggesting *H. pylori* has glycoproteins throughout its cellular structure. Moreover, each of these fractions displayed a different glycoprotein profile, suggesting that *H. pylori* glycosylates proteins with varying biological roles. However, it is important to note that some differences in subcellular glycoprotein profiles were observed between the two sets of fractions. These differences may be attributable to phase-variation between the *H. pylori* cultures used to prepare the two sets of fractions. Future work will seek to replicate one of the glycoprotein profiles already observed.

The anti-FLAG immunoblots of both fraction sets displayed two distinct bands representing secreted glycoproteins. Since secreted proteins are often virulence factors and/or facilitators of host-pathogen interactions, I sought to identify these two glycoproteins through mass spectrometry, which requires a relatively pure glycoprotein sample. Several attempts were made to enrich the secreted fractions for glycoproteins using anti-FLAG and nickel affinity chromatography (which bind the FLAG and His₆ moieties of Phos-FLAG-His₆, respectively). However, these attempts were unsuccessful in obtaining an enriched glycoprotein sample. It appears likely that low starting concentrations of...
glycoproteins relative to total protein, or competitive binding from unreacted Phos-FLAG-His, was responsible. Due to the challenges encountered, future attempts at identifying glycoproteins will seek to profile other fractions before returning to the secreted fraction.

In the hopes simplifying the purification process, I have also recently completed the synthesis of a new phosphine-conjugated bead. This bead should allow for the covalent capture of azide-labeled glycoproteins from cellular fractions via a solid-phase Staudinger ligation. Following extensive washing to remove other proteins, the glycoproteins can be released from the bead via an incorporated safety-catch linker. I hope to test the efficacy of this bead by using SDS-PAGE to analyze purified whole-cell lysates relative to unpurified lysates. If this bead proves to be effective, it will make the purification and analysis of azide-labelled glycoproteins from cellular fractions much faster and simpler.

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**References**