Investigating optimal design parameters for bacterial uptake of monosaccharide probes

Jack Tran '26

Bacteria are covered with a complex coat of monosaccharides known as glycans, which help facilitate crucial bacterial functions, including motility, cell adhesion, and pathogenicity.[1] The rise of antibiotic-resistant bacteria has become a more pressing issue in the pharmaceutical community over the last few decades, and alternative solutions are needed to address this crisis. These bacterial glycans are an attractive potential target for developing bacterium-specific antibiotics because the glycan composition of bacteria is extremely diverse, with glycans being composed of over 700 unique monosaccharide building blocks.[2]

However, due to the structural diversity, studying these glycans and their role in causing disease is a difficult task. One method to solve this problem involves utilizing monosaccharide probes, or molecules that are nearly identical to those in the bacterial glycans, containing a small modification that allows them to be tracked. This modification was the addition of azide groups on the sugar, a functional group with three nitrogen atoms that react selectively and bind with an energy-packed triple bond in a fluorescent cyclooctyne molecule.[3] Briefly, the bacterial cells were supplemented with the azide-sugar analogs to allow for glycan incorporation. Then, the fluorescent reporter molecule was added, reacting with the azide sugars on the bacterial membrane and allowing for detection and quantification of total probe incorporation.

For my research project, I investigated peracetylation as an additional modification to these monosaccharides as a way to ease initial uptake into the bacterial membrane. Discovering ways to promote the incorporation of monosaccharide probes is a significant step in the long-term goal of developing bacterium-specific antibiotics, as these antibiotics will likely contain similar modifications. Peracetylation was hypothesized to increase incorporation because the bacterial cell membrane is nonpolar, and replacing the polar hydroxyl groups with nonpolar acetyl groups would decrease probe polarity, making it easier to cross the membrane. A panel of bacteria was exposed to both the peracetylated and free sugar containing the azide group, and reacted with the fluorescent reporter molecule. Then, total fluorescence was measured to determine the amount of incorporation. It was revealed that for N-acetylglucosamine and N-acetylgalactosamine, two monosaccharides commonly found in bacterial glycans, peracetylation of the sugars led to significantly increased uptake in *Helicobacter pylori*, *Plesiomonas shigelloides*, and *Vibrio vulnificus* when compared to the free sugar.

These findings were significant because they demonstrated that peracetylation is a viable method to promote incorporation of monosaccharide probes into bacterial glycans. Further research will investigate if the discovered trend remains consistent for additional azide sugars, as well as in other bacteria. Further, future research could investigate the use of other nonpolar functional groups instead of peracetylation to determine if there is a more viable alternative.

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References

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