Therapeutic targeting of pathogenic bacteria via copper-catalyzed click chemistry

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The efficiency of modern antibiotics in selectively eradicating infectious bacteria has declined significantly due to the emergence of antibiotic resistance bacterial strains [1, 2] and the adverse disruption of the human microbiome [3, 4]. Unique sugar-modified proteins termed glycoproteins have presented a novel target for therapeutic intervention due to their presence on bacterial cell surfaces [5], their differential expression across mammalian and bacterial species [6], and their linkage to pathogenicity and survival [7]. Due to the diversity of these sugar-protein structures, glycoproteins offer a potential target for selective covalent therapeutic compounds, and could enable pathogen eradication without risking the health of the host microbiome [8]. Helicobacter pylori, a Gram-negative bacteria, serves as an example of a pathogen with increasing resistance to its present therapeutic approach whose glycoproteins can be targeted with covalent therapeutics [8].

$H.~pylori$ has infected over 50% of the world’s human population and is primarily associated with symptoms such as duodenal ulcers, gastritis, and gastric cancer [2].

Metabolic oligosaccharide engineering (MOE) has been employed in previous studies in the Dube lab to selectively label and target $H.~pylori$’s glycoproteins for detection and therapeutic intervention [9]. This technique labels bacterial glycoproteins with abiotic monosaccharides via endogenous metabolic pathways, providing distinct targets for therapeutic agents that do not interact with common biological functional groups [10]. Azides present on bacterial glycans can undergo bioorthogonal reactions to enable the successful delivery of phosphine-, cyclooctyne-, or alkyn-based probes or therapeutic agents. The Dube lab has demonstrated the successful reaction between azide-labeled glycans and phosphine- and cyclooctyne-based probes via Staudinger ligation [11, 12] and strain-promoted copper-free click chemistry [12, 13] respectively. However, these reactions suffer from sluggish reaction rates and tendencies to participate in non-specific side-reactions, diminishing their potential clinical utility [12].

To circumvent these drawbacks to current therapeutic agents, a novel covalent compound was designed with the potential to undergo copper-catalyzed click chemistry, a bioorthogonal reaction with faster reaction rates and significantly higher selectivity [14]. Previously, copper-catalyzed click chemistry was avoided due to the cytotoxic effects of the copper catalyst. However, recent studies have developed copper chelating ligands that have reduced the detrimental effects of copper on cell viability, revitalizing their utility for biochemical uses [15, 16]. The Dube lab aims to circumvent the issues of current azide-reactive partners by utilizing an alkyn functional group to participate in copper-catalyzed alkyn-azide cycloaddition with azide-labeled glycans. This summer, I synthesized a conjugate bearing the FLAG peptide, an alkyn functional group that could partake in azide-alkyne cycloaddition to form triazole products, and the immune stimulant DNP, which could elicit cell death in the presence of immune effector cells. Successful synthesis of K(DNP)-Alkyne-FLAG and its purity were confirmed via High-Performance Liquid Chromatography (HPLC) and Liquid-Chromatography/Mass-Spectrometry (LC/MS).

Copper-catalyzed click chemistry necessitates a copper source in order to lower the activation energy enough to produce appreciable rates of the triazole compound. Copper was provided to the azide-alkyne reaction by reducing copper sulfate with sodium ascorbate in situ [17]. Aminoguanidine and an accelerating ligand were also incorporated to prevent the production of reactive oxygen species and undesired glycation endproducts [18, 19]. The ability of K(DNP)-Alkyne-FLAG to react with azide-labeled bacterial lysates was assessed via western blot analysis. Such analysis demonstrated that the synthesized K(DNP)-Alkyne-FLAG reacts with azide-labeled $H.~pylori$ without similarly labeling azide-free controls, contrasting results seen with previous cyclooctyne-based reactive-partner conjugates.

In future work, the ability of K(DNP)-Alkyne-FLAG to eradicate azide-labeled bacteria as compared to that of other DNP-conjugated therapeutics previously used in the Dube lab (BCN-PEG3-CK(DNP)-FLAG, Phos-K(DNP)-FLAG, DIBAC-K(DNP)-FLAG), will be assessed by immune-mediated killing assays. Similar assays will then be conducted with photodynamic therapeutic PpIX-Alkyne-FLAG [20]. Lastly, the administration of copper in therapeutic approaches limits the clinical utility of copper-catalyzed click chemistry due to the adverse health effects of copper accumulation in the human intestinal system [21]. Therefore, future protocols will incorporate newly-developed chelator bis(tert-butyltriazoyl) ligand (BTTES) that catalyzes alkyn-azide cycloaddition at appreciable rates without effecting the viability of host cells [16].

Research Mentor: Danielle H. Dube, Department of Chemistry & Biochemistry
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References


