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Structure-function analysis of the gonococcal TonB-dependent transporter, HpuB

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Background

Neisseria gonorrhoeae (Ngo), is a “superbug” that impacts millions of people worldwide. A gonococcal infection does not lead to protective immunity, there is no effective vaccine to date, and antimicrobial resistance is increasing; this urgent threat requires novel treatment strategies and vaccines. During nutritional immunity, the human host sequesters iron away from invading pathogens to prevent bacterial growth and pathogenesis. To overcome nutritional immunity, Ngo has evolved bipartite transport systems that recognize the host’s iron-sequestering proteins as ligands. These proteins, such as hemoglobin (Hb), bind to extracellular loops of the surface-exposed outer membrane transporters, making these transporters promising targets for the development of vaccines or therapeutics. Two required proteins make up the hemoglobin utilization system: HpuA lipoprotein, and HpuB the transporter.

Aim/Methods

We hypothesized that deleting extracellular loop regions of hpuB or conserved motifs in hpuB putatively responsible for heme uptake would impair the ability of Ngo to bind Hb and internalize iron. To test this hypothesis, we inactivated the native copy of hpuB using an omega cassette and placed the mutated or wild-type (WT) copy of hpuB behind a lac promoter in an ectopic site on the gonococcal chromosome. Next, using ELISA, we tested the binding ability of these mutants and grew these strains, with Hb as a sole iron source, to test their ability to internalize iron.

Results

HpuB expression by the mutant strains was verified utilizing whole cell lysates prepared from cells grown with or without IPTG; a protease accessibility assay confirmed surface exposure of HpuB in the mutants. Employing a growth assay with Hb as a sole iron source, we identified a few hpuB mutations that impaired growth when the lipoprotein partner, HpuA, was insertionally inactivated. Some of the mutants were also deficient in Hb binding when HpuA was non-functional as assessed by an ELISA assay using HRP-conjugated Hb.

Conclusions

Together, these findings highlight critical residues in HpuB that are important for Hb binding and iron utilization. This work will help better characterize the HpuAB utilization system and may consequently help the search for an effective vaccine.