Identification of mucosal glycans that regulate the acute-to-chronic infection switch in *Pseudomonas aeruginosa*

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The opportunistic human pathogen *Pseudomonas aeruginosa* is the leading cause of morbidity and mortality in immunocompromised patients and in patients suffering from cystic fibrosis. *P. aeruginosa* causes acute infections that spread rapidly causing tissue damage and sepsis with high mortality rates, and chronic infections that persist for extensive periods requiring intensive clinical intervention.[1] Recently, it was shown that mucin *O*-glycans can directly suppress virulence-associated traits in *P. aeruginosa,* including both acute and chronic infection, without affecting bacterial survival.[2] This ability to attenuate virulence represents a promising therapeutic approach with reduced pressure to develop drug resistance. However, the molecular mechanisms by which glycans afford this effect are unclear.

RetS and LadS are antagonist sensor kinases that control the acute-to-chronic infection switch in *P. aeruginosa*. These sensors are composed of a cytoplasmic histidine kinase, a 7-transmembrane region (7TMR), and a periplasmic sensor domain DISMED2 (diverse intracellular signaling module extracellular 2). LadS stimulates chronic infection behavior, while RetS promotes acute infection.[3] It has been shown that glycans associated with mucin glycoproteins isolated from mucus downregulate genes involved in chronic infection. This effect is absent in *P. aeruginosa* (PA14) RetS knock-out mutants and RetS DISMED2 mutants.[4] The crystal structure of RetS DISMED2 reveals two putative glycan binding sites. One with high similarity to an analogous site in structurally-related carbohydrate binding modules (CBMs), and a second with no similarity to CBMs.[5,6] A structural analysis of LadS DISMED2 domain reveals conservation of only one ligand binding site (CBM-like). These features suggest that RetS and LadS could be involved in mucin glycan sensing and virulence attenuation. In our work, we recombinantly produced the DISMED2 domains of RetS and LadS and identified the specific *O*-glycan structures recognized by each. In addition, we performed site-directed mutagenesis at the ligand binding sites to identify the amino acids involved in glycan binding. These results contribute to the characterization of the molecular mechanism behind *O*-glycan pathogenicity attenuation and the role of RetS and LadS sensor kinases, aiming to develop novel glycan-based therapeutics.

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1. Turner, K. H., Everett, J., Trivedi, U., Rumbaugh, K. P. & Whiteley, M. Requirements for Pseudomonas aeruginosa Acute Burn and Chronic Surgical Wound Infection. *PLoS Genet.* **10**, e1004518 (2014).
2. Wheeler, K. M. *et al.* Mucin glycans attenuate the virulence of *Pseudomonas aeruginosa* in infection. *Nat. Microbiol.* **4**, 2146–2154 (2019).
3. Goodman, A. L. *et al.* Direct interaction between sensor kinase proteins mediates acute and chronic disease phenotypes in a bacterial pathogen. *Genes Dev.* **23**, 249–259 (2009).
4. Wang, B. X. *et al.* Mucin Glycans Signal through the Sensor Kinase RetS to Inhibit Virulence-Associated Traits in *Pseudomonas aeruginosa*. *Curr. Biol.* **31**, 90-102.e7 (2021).

5. Vincent, F. *et al.* Distinct oligomeric forms of the *Pseudomonas aeruginosa* RetS sensor domain modulate accessibility to the ligand binding site: Structure of the RetS sensor domain. *Environ. Microbiol.* **12**, 1775–1786 (2009).
6. Jing, X., Jaw, J., Robinson, H. H. & Schubot, F. D. Crystal structure and oligomeric state of the RetS signaling kinase sensory domain. *Proteins Struct. Funct. Bioinforma.* NA-NA (2010) doi:10.1002/prot.22679.