Biophysical Investigations Into the Structure and Function of Pseudomonas aeruginosa Nitric Oxide Signaling System

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Nitric oxide sensing proteins (NosPs) have been studied as important signaling molecules in various organisms. Particularly in Pseudomonas aeruginosa, NosP plays a role in heme binding and controlling the phosphorylation of NosP's associated histidine kinase, NahK. Recently, heme binding has been of particular interest and how this is associated with the signaling pathways in bacterial biofilm formation. Computational structural characterization of P. aeruginosa NosP predicts that heme binding occurs between the tri-symmetrical base and disordered cap regions. The cap is believed to open and secure the heme in position. Despite these predictions, the role of NosP's cap remains poorly uncharacterized. As a result, it was hypothesized that generating a NosP construct without the cap (?208-301) will diminish kinase inhibition and heme binding during in vivo and in vitro testing. To test this, capless, tagless, NosP was generated and purified, and the apo, ferric, and ferrous complex ligation states were assessed with UV-Vis spectroscopy. The inhibitory potential of these complexes with NahK was tested using HPLC analysis. The phenotypes downstream of NahK, such as the production of pyocyanin and swarming motility, were checked to see if the cap affects the ability of it to inhibit NahK. Interestingly, the in vivo data shows strong phenotypic evidence of the capless NosP exhibiting tighter inhibition, while the preliminary investigation into the capless mutant’s heme binding properties show altered mechanics and reduced inhibition in vitro. Therefore, future work is needed to further understand how the heme binding plays a role in kinase inhibition.

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