Biophysical Characterization of the Pseudomonas aeruginosa BqsR/BqsS Two-Component System

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Pseudomonas aeruginosa, a ubiquitous Gram-negative bacterium best known for infecting the lungs of cystic fibrosis (CF) patients, is one of the major causes of chronic nosocomial infections and can grow either as planktonic or biofilm. Biofilms are complex microbial structures capable of providing an advantageous protective quality that causes bacteria living within a biofilm to be significantly more resistant to antibiotic treatments than planktonic bacteria, representing a major health threat. Recent studies have uncovered a novel two-component signal transduction system that regulates biofilm formation/decay in *P. aeruginosa* through extracellular Fe^{2+} binding known as BqsR/S. The sensing of Fe^{2+} is important, as this nutrient is present throughout each stage of infection in CF sputum and constitutes a large portion of the iron pool present in advanced stages of lung function failure. *Pa*BqsS has been identified as a transmembrane sensor kinase while *Pa*BqsR has been identified as a cytosolic response regulator that binds to DNA and is capable of altering transcription of genes involved in biofilm formation. However, neither of these proteins have been structurally characterized, and the details of how and to what extent they interact with Fe^{2+} remain unknown. In this work, we have expressed, purified, and initially characterized *Pa*BqsR using NMR structural techniques. These results reveal the presence of a well-folded, monomeric protein in the absence of phosphorylation. Using X-ray crystallography, we have solved the structure of the N-terminal phosphorylation domain of *Pa*BqsR to 1.3 Å resolution, revealing a canonical $(\beta\alpha)_5$ response regulator assembly that consists of a central five-stranded parallel β-sheet surrounded by five helices. Surprisingly, generation of two phosphorylation mimics (a D51E *Pa*BqsR variant and a BeF_3^--incubated form of *Pa*BqsR) maintains the monomeric oligomerization of the response regulator based on gel filtration studies. Interestingly, we have found that the reported *Pa*BqsR DNA-binding consensus sequence is located upstream of the *feo* operon, the primary Fe^{2+} transporter of *P. aeruginosa*. Preliminary electrophoretic mobility shift assays (EMSAs) show that *Pa*BqsR is indeed capable of binding upstream of the *feo* operon, but only in the pseudo phosphorylated state. Additionally, we have created a protocol that produces highly pure, monodisperse detergent-solubilized *Pa*BqsS and have investigated its ability to bind metal. Based on previous studies that indicated a periplasmic RExxE motif in *Pa*BqsS may bind metal, we used site-directed mutagenesis experiments to modify the RExxE motif, and we tested the efficacy of WT and variant *Pa*BqsS to bind Fe^{2+}. WT *Pa*BqsS indeed binds Fe^{2+} with a stoichiometry of 1 Fe^{2+} ion per dimer. The *Pa*BqsS RAxxE variant binds Fe^{2+} the same as WT, while the RExxA variant completely loses the ability to bind Fe^{2+}. These results are indicative of binding at a dimer interface at Glu^{48}, possibly in a tetrahedral geometry. These results provide the first biophysical characterization of the Bqs system and demonstrate an unexpected connection between the BqsR/S system and the Feo system, an important *P. aeruginosa* virulence factor.