Investigation of the Role of W66 in Heme Ruffling in IsdI

Staphylococcus aureus, a highly virulent pathogen, has gained significant attention due to the emergence of various antibiotic resistant strains, including methicillin resistant *S. aureus* (MRSA) and vancomycin resistant *S. arueus* (VRSA).[1] One new drug target involves the inhibition of the Iron Surface Determinant (Isd) pathway which scavenges Fe^{3+} from hemoglobin.[2] Specifically, IsdI and IsdG, two heme-oxygenases (HO) involved the catalytic cleavage of an iron-containing tetrapyrrole, heme,[2] are of interest. These two enzymes, unlike eukaryotic HO's, have been show to bind heme in a ruffled manner potentially producing a novel cleavage of Heme by the bound peroxide molecule.[3] The active site residue W66 in IsdI, absent in eukaryotic HO's, has been implicated in this heme ruffling through x-ray crystallography.[4] Additionally, the W66A variant of IsdI is inactive.[3] Through magnetic circular dichroism (MCD) spectroscopy and ^{1}H nuclear magnetic resonance (^{1}H-NMR) spectroscopy coupled with electronic structure calculations the geometric and electronic structure of wildtype and W66A IsdI can be analyzed and compared. In order to emulate the peroxide bound species, which is much too reactive to be isolated, N_3^- and CN^- bound forms were prepared. The MCD spectra of these forms of wildtype IsdI have been collected. These spectra will be compared with the N_3^- and CN^- bound forms of W66A IsdI species in order to determine how the mutation affects the heme electronic structure and triggers IsdI inactivation.

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