The Met-Tyr-Trp Crosslink in *Mycobacterium tuberculosis* Catalase-Peroxidase (KatG): A Structure-Function-Spectroscopy Relationship

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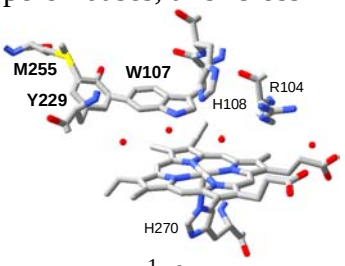
Catalase-peroxidases (KatG) are bifunctional enzymes possessing both catalase and peroxidase activities. Three crystal structures of different KatGs each revealed the presence of a novel active site modification comprised of two covalent bonds between three amino acid side chains: Trp107, Tyr229, and Met255 (*Mycobacterium tuberculosis* numbering). Absent from the peroxidases, this 'crosslink' has been suggested to impart catalatic activity to the KatGs.



In order to better understand the role which the crosslink plays in enzyme catalysis, we have studied recombinant *Mtb* WT KatG and two mutants, KatG(Y229F) and KatG(M255I). LC-MS studies confirm the presence of the Met255-Tyr229-Trp107 crosslink in WT KatG, whereas KatG(M255I) exhibits only one covalent bond (Tyr-Trp), and KatG(Y229F) lacks both linkages. Catalase activity was lost in the two mutants (k_{cat}: 0.1 and 1.1 s^{-1} for Y229F and M255I, vs. 6000 s^{-1} for WT), whereas peroxidase activity was enhanced (k_{cat}: 0.843 and 0.164 s^{-1} for Y229F and M255I, vs. 0.062 s^{-1} for WT). Optical stopped-flow studies revealed an unusual compound II spectrum for WT KatG [UV-vis: 410 (Soret), 628 nm], best described as $(P\bullet)Fe^{III}$, where P• represents a protein-based radical. This contrasts with the 'classical' oxoferryl compound II spectrum observed for KatG(Y229F) [UV-vis: 417 (Soret), 531, 561 nm].

WT KatG lacking the Met-Tyr-Trp crosslink was also prepared, making possible studies of its formation under oxidizing conditions. Incubation of this 'crosslink-free' KatG with various peracids led to crosslink formation via compound I, the $(Por^{\bullet+})Fe(IV)$=oxo intermediate, but not by compound II, and a mechanism for Met-Tyr-Trp autocatalytic formation will be presented.

We have been able to show that the structural element comprised of the crosslinked Met-Tyr-Trp amino acids forms in an autocatalytic process, that its presence alters the spectral features of compound II, and that this correlates well to changes in enzymatic function, particularly catalase activity. It is clear from this work that an enzyme structure-spectroscopy-activity relationship is readily observed in the catalase-peroxidases which is solely attributed to the presence of the Met-Tyr-Trp crosslink. These and other supporting results will be presented.