High-Field EPR Study of the Novel MetTyrTrp Cofactor Radical in Catalase-peroxidase

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Introduction

Catalase-peroxidase (KatG) is a ubiquitous enzyme in aerobic microorganisms, which in the human pathogen *M. tuberculosis* catalyzes the detoxification of H_2O_2 *($2H_2O_2 \rightarrow 2H_2O + O_2$).* Here we focus on characterizing the radical localized on the unique Met-Tyr-Trp (MYW) adduct [1] found in all KatG enzymes as the steady-state species present during the catalase reaction [2]. This chemistry is entirely new in the enzymology of heme/radical enzymes.

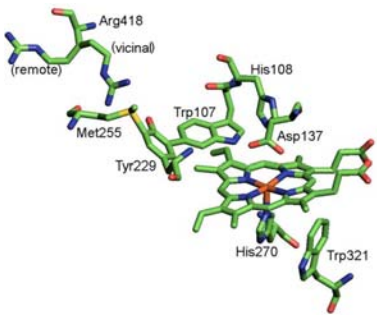

Figure 1. The heme pocket and surrounding structure in *M. tuberculosis* catalase-peroxidase (KatG, 2cca.pdb) showing the MYW adduct and the two conformers of the Arg418 side chain.

Experimental

Samples were prepared in the PI's lab by mixing excess H_2O_2 with resting (Fe(III)KatG followed by rapid freezing in liquid ethane to produce and freeze-quench the MYW-adduct radical intermediate. Spectra were recorded over range of temperatures and at multiple frequencies at the EMR Facility of the NHMFL using the 15/17 T superconducting magnet.

Results and Discussion

The HFEPR spectra for both wild-type, and Arg418Leu mutant are shown in Figs. 2 and 3, respectively. A small difference in the g_x-value for the MYW-adduct radical was found for the inactive Arg418Leu mutant compared to wild-type KatG (2.0050 vs. 2.0063).

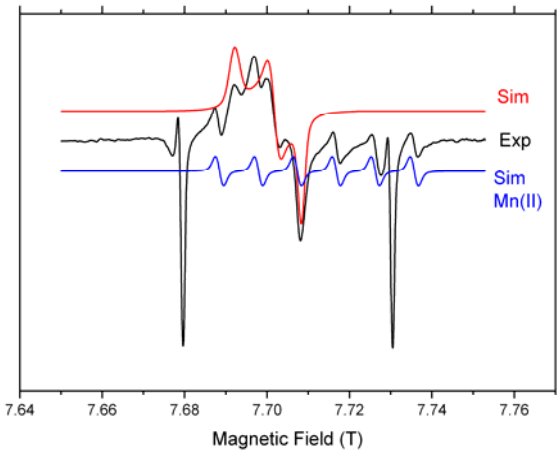

Figure 2. EPR spectrum of the Met-Tyr-Trp adduct radical species trapped during the catalase reaction of *M. tuberculosis* KatG, recorded at 216 GHz and 15 K (black trace). Simulation (red trace, after subtraction of Mn(II) spectrum, blue trace) used g = [2.0063, 2.0038, 2.0021]

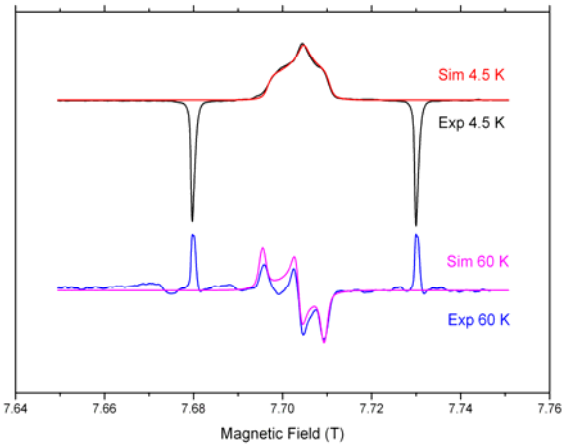

Figure 3. EPR spectrum of the Met-Tyr-Trp adduct radical in the Arg417Leu mutant trapped during the catalase reaction of *M. tuberculosis* KatG, recorded at 216 GHz and 4.5 K (black) or 60 K (blue). Simulation at 4.5 K (red trace) used *g* = [2.0050, 2.0029, 2.0014] while that at 60 K (magenta) used *g* = [2.0053, 2.0032, 2.0017]

Conclusions

Differences in hydrogen bonding between the Arg and the Tyr residue within the adduct radical can explain the lost function in the Arg418Leu mutant enzyme.

Acknowledgements

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References

[1] Suarez, J., *et al.*, J. Biol. Chem. **284**, 7017-7029 (2009).
[2] Zhao, X., *et al.*, J. Am. Chem. Soc. **132,** 8268-8269 (2010).