Structure-function relationship in heme oxygenase from *Mycobacterium tuberculosis* - experimental evidence for new oxidizing intermediate

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Mycobacterium heme utilization degrader (MhuD) is a non-canonical heme oxygenase that plays a critical role in the heme uptake pathway in *Mycobacterium tuberculosis* (Mtb) and has been identified as auspicious antitubercular drug targets [1]. MhuD is distinguished from canonical heme oxygenase enzymes by its heme degradation products, releasing mycobilins and ferrous iron without generating carbon monoxide [1]. Interestingly, MhuD can bind one or two heme molecules in the same active site. Although it was initially proposed that monoheme MhuD is enzymatically active and diheme is inactive [1], our recent work showed that, actually, both forms can effectively degrade heme [2]. The mechanism of heme degradation is complex and controversial, with ferric hydro/peroxo (Fe(III)-O-O(H)) or ferryl heme (Fe(IV)=O) species being proposed as reactive intermediates [3,4]. Resonance Raman (rR) spectroscopy is an ideal technique to probe the active site architecture of heme proteins not only in their stable but also in fleeting intermediates states. Employed in this work combination of rR spectroscopy and cryoradiolysis methodology revealed convincing experimental evidence that the second reduction in the enzymatic cycle of MhuD occurs at the Fe atom rather than the dioxygen moiety, forming a ferrous superoxo intermediate instead of the expected ferric peroxo species. This species has been proposed to exist in equilibrium with ferric superoxo porphyrin anion radical (FSPAR) intermediate, which DFT modeling showed earlier to be associated with a ruffled heme of model compounds [4]. Altogether, the combination of several powerful biophysical and biochemical strategies allows for redefining the functional paradigm of diheme MhuD and expanding its role in Mtb metabolism.

REFERENCES

1. Chim, N.; Iniguez, A.; Nguyen, T. Q.; Goulding, C. W. *J. Mol. Biol*. **2010**, *395*, 595-609.
2. Snyder, N. S.; Mak, P. J. *J. Biol. Chem*. . **2022**, *298*, 101475-101490.
3. Matsuia,T.; Nambu, S.; Goulding, C. W.; Takahashi, S.; Fujii, H., Ikeda-Saitoa, M. *Proc. Natl. Acad. Sci. U. S. A*. **2016**, *113*, 3779-3784.
4. Ohta, T.; Liu, J.-G.; Nagaraju, P.; Ogura, T.; Naruta, Y. *Chem. Commun*. **2015**, *51*, 12407-12410.