MS09-1-2 Myoglobin as a model for heme peroxidase intermediate determination via integrative structural biology
#MS09-1-2

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Abstract
Heme-peroxidases were amongst the earliest enzymes investigated, and function to catalyse a breadth of substrates in a H2O2- dependent oxidation reaction. The progress of their reactions can be monitored by observing the electronic absorption spectra in the near UV-visible region as well as other indirect techniques. The distinct spectral signatures are due to structural and electronic changes at the heme moiety iron centre(1). Two major intermediate states, Compounds I and II, have iron oxidised above the resting Fe(III) “ferric” state to Fe(IV)-oxo “ferryl” state. Whilst readily identifiable via spectroscopic measurements, crystallographic and spectroscopic determinations of their precise nature is often conflicting. As the protonation state of these intermediates, being protonated (Fe(IV)–OH) or deprotonated (Fe(IV)=O), directly determines the enzymes reactivity, it is therefore apropos to correctly characterise them (2).
Despite not being a formal peroxidase, myoglobin is an excellent model as it exhibits low level basal peroxidase activity with analogous ferryl intermediates that can be monitored spectroscopically (3-5). Furthermore myoglobin readily forms high resolution crystals, has been used as a model system for the development of serial crystallography (6-8) and forms stable long-lived intermediates including Compound II. Our research aims to take an integrative structural biology approach to unequivocally address the ambiguity surrounding the peroxidase intermediates of myoglobin. Historically, the Fe-O bond length was taken as a proxy as bond order, however accurately measuring bond lengths with the precision needed with traditional X-ray diffraction, and being confident of avoiding X-ray induced photoreduction (despite multicrystal analyses and cryo-cooling) does not give the certainty of direct observation that is available via high resolution neutron crystallography, or X-ray free-electron laser (XFEL) diffraction. Similarly, the rise of microcrystal electron diffraction (microED) and the Coloumbic potential maps generated would permit direct observation of charge states, and facilitate a proof of principal for a pump-probe experiment at SFX-XFEL sources. Accumulation of a reaction intermediate has be validated with in crystallo and solution state UV-spectroscopy. Combining our solution spectroscopic results with the precise hydrogen positioning of a perdeuterated crystal amenable to neutron diffraction would begin to clarify the heme site protonation state. Additionally, microcrystalline slurries of hydrated crystals interrogated with microED and XFEL crystallography would incorporate charge potentials and radiation damage free structures surrounding the heme centre, respectively, supplementing the neutron studies. Integrating these results would build an intimate understanding of the chemistry driving this model peroxidase. Our group has previously collected Neutron and XFEL diffraction of two formal peroxidases (cytochrome c peroxidase and Ascorbate Peroxidase). With the Compound II intermediate protonation states appearing to contrast one another in these systems (9-11), describing the heme chemistry which drives the peroxidase activity within myoglobin succinctly using this integrative approach, will address a four decade long question and deepen our understanding of heme peroxidases.

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