What Prevents Non-Heme Iron Halogenases from Performing Hydroxylations?

Kevin Harnden Literature Seminar September 16, 2014

Since 1938 there are more than 1500 unique compounds approved as drugs by the FDA. Of these about 15 percent are organochlorine compounds with another 3 percent organobromine or organoiodine compounds.[1] The halogenation of synthetically produced compounds has been shown to increase their efficacy in certain cases.[2] The halogenation of both synthetic and biological products has gained interest recently due to the applications for medicinal and environmental chemistry. In biological systems the halogenation of hydrocarbons is catalyzed by various types of enzymes, which can be classified as either haloperoxidases or halogenases. Compared to the haloperoxidases, the halogenases are less studied, due to their more recent discovery less than ten years ago, and able provide a greater degree of selectivity with regards to the production of a specific target compound.[3] Out of the three classes of halogenases, the non-heme iron containing class is the only one capable of halogenating unactivated alkanes. For this reason, the non-heme iron halogenases have been the subject of significant investigation.

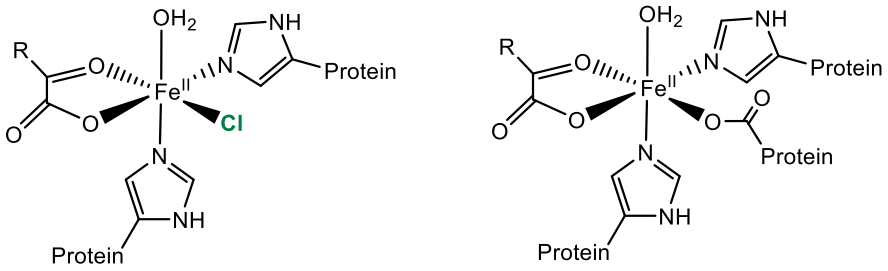

Figure 1. Active site of non-heme iron halogenase (left) and monooxygenases (right).

Non-heme iron halogenases are structurally similar to another class of enzymes known as α-ketoglutarate dependent non-heme iron monooxygenases (Figure 1).[4] These monooxygenases contain an iron(II) coordinated by a conserved 2-His/1-carboxy iron binding motif, composed of two are histidines, either a glutamate or an aspartate from the protein, and a bidentate cofactor known as a-ketoglutarate. Non-heme iron monooxygenases activate molecular oxygen to form an iron(IV)-oxo, which is capable of oxidizing aliphatic substrate to produce a substrate radical (Figure 2). This radical will rapidly react with the hydroxyl ligand of the iron resulting in an alcohol. Non-heme iron halogenases have an identical ligand set with the exception of a halide ligand in the place of the protein carboxylate. The catalytic cycle of halogenases and monooxygenases is similar until the final step in which halogenases will selectively produce the halogenated product.

There have been several predictions, based on computational studies, put forward in an attempt to explain this selectivity in halogenation. These predictions include the protonation of the hydroxyl ligand of the iron(III) by the surrounding amino acids after the formation of the substrate radical[5], diminishing the ability for hydroxylation to occur, and

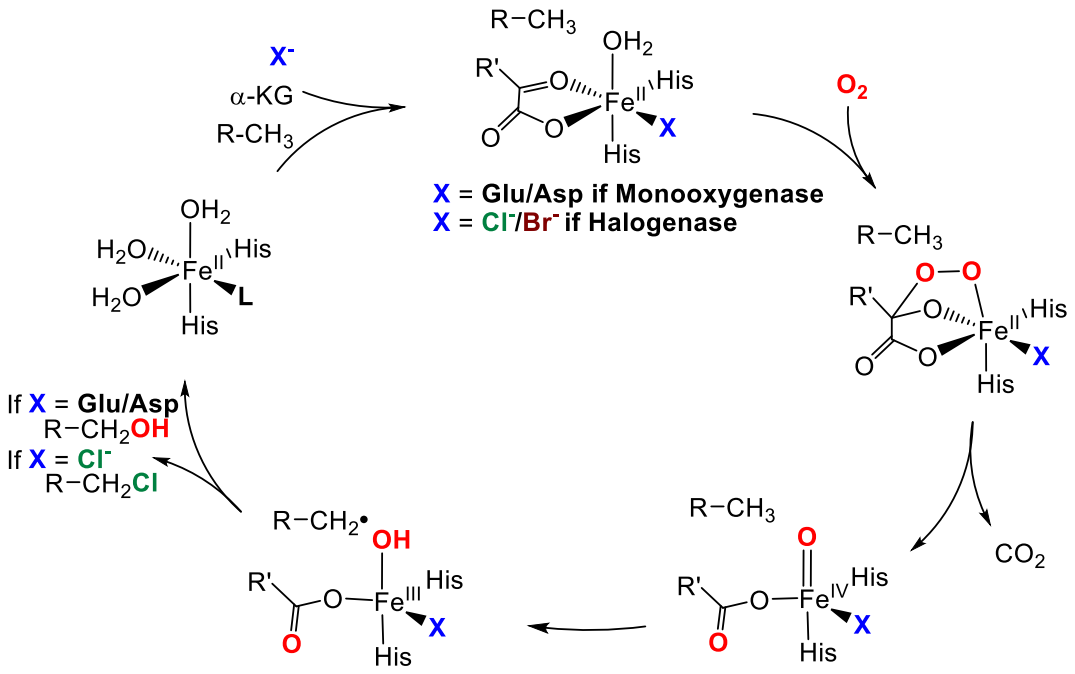

Figure 2. Catalytic cycle of non-heme iron halogenases and monooxygenases.

the formation of bicarbonate via the nucleophilic attack of the hydroxyl ligand on the carbon dioxide released during the catalytic cycle[6], eliminating the ability for hydroxylation. While these have support from computational work, there has been no experimental work done to confirm this as a way for the enzymes to control reactivity. Other predictions have been tested experimentally and shown to play a tentative role in the selective halogenation. These include ligand based conformational changes as a way to gate the progression of the reaction[7], and another where the careful position of the substrate relative to the iron site allows for the selective formation of the halogenated product.[8] More specifically, molecular dynamic simulations show that the iron site could have a third ligand from the protein, a glutamate, in addition to the two histidines. This glutamate is able to rotate out of a coordinating position if it is not bound to the iron, causing a conformational change to occur. The other proposed method of selectivity was shown by positioning the substrate radical in different sites relative to the iron(III), which was found to change the activation energy for both hydroxylation and halogenation pathways, and as a result change which reaction was favored.

In order to explore these two explanations, the catalytic cycle can be separated into two phases. The first phase takes place before oxygen activation involves and the association of the chloride ligand. The second phase takes place after oxygen activation and involves hydrogen atom abstraction of the substrate by the iron(IV)-oxo intermediate. In each phase of the catalytic cycle, the enzyme must control the reactivity in order to selectively produce the halogenation product.

In the first phase, the addition of chloride to the solution is expected to induce a conformational change in the enzyme. Studies show that oxygen is not consumed until chloride is added.[4] To investigate further, variable temperature, variable field magnetic circular dichroism (VTVH MCD) was used to elucidate the effect of chloride on the iron

electronic state.[7] Chloride was found to be necessary in order for the coordination number of the iron to change from six to five, which will provide an open binding site for oxygen. This finding supports the predicted model where association of chloride induces a conformational change.

In the second phase, simulations have shown that the activation energy for both pathways, halogenation and hydroxylation, will change based on the position of the substrate radical relative to the ligands of iron.[8] This was shown experimentally by treating the enzyme with non-native substrate, the hydroxylation product formed as opposed to the halogenation product normally produced.[9] By examining the position of the non-native substrate in the active site, it was shown to be positioned closer to the hydroxyl ligand and further away from the chloride ligand. To investigate this further, the iron(IV) intermediate was trapped at low temperatures and characterized using nuclear resonance vibrational spectroscopy (NRVS).[10] Using this method, the stretches and bends associated with only an ^{57}Fe are observed so the iron site can be studied while ignoring the rest of the protein. By fitting the observed spectrum with the simulated spectrum of iron complexes with varying geometries, the iron(IV) intermediate was found to be trigonal bipyramidal with the oxo ligand axial and the chloride ligand equatorial. When this model is placed in the crystal structure the iron(IV)-oxo is found to be in a position to perform a hydrogen atom abstraction of the substrate using the π^* orbital of the oxygen. Once the substrate radical forms the chloride ligand is now closer than the hydroxyl ligand, allowing for the selective addition of chlorine to the substrate.

What prevents non-heme iron halogenases from performing hydroxylations? Overall, these non-heme iron halogenases selectively produce the halogenated product using chloride based conformational changes, which lead to an active enzyme that can activate molecular oxygen, and by orientating the substrate in such a way that the iron(IV)-oxo is capable of performing the necessary hydrogen atom abstraction while the chlorine is in a position to add to the substrate radical, selectively produces the halogenated product.

References

1. Wilcken, R.; Zimmermann, M. O.; Lange, A.; Joerger, A. C.; Boeckler, F. M. *J. Med. Chem.* **2013**, *56*, 1363-1388.
2. Gribble, G. W. *J. Chem. Educ.* **2004**, *81*, 1441-1449.
3. Butler, A.; Sandy, M. *Nature*. **2009**, *460*, 848-854.
4. Mathews, M. L.; Chang, W.; Layne, A. P.; Miles, L. A.; Krebs, C.; Bollinger, J. M. *Nat. Chem. Biol.* **2014**, *10*, 209-215.
5. Pandain, S.; Vincent, M. A.; Hillier, I. H.; Burton, N. A. *Dalton Trans.* **2009**, *38*, 6201-6207.
6. de Visser, S. P.; Latifi, R. *J. Phys. Chem. B.* **2009**, *113*, 12-14.
7. Pratter, S. M.; Light, K. M.; Soloman, E. I.; Straganz, G. D. *J. Am. Chem. Soc.* **2014**, *136*, 9385-9395.
8. Kulik, H. J.; Drennan, C. L. *J. Biol. Chem.* **2013**, *288*, 11233-11241.
9. Matthes, M. L.; Neumann, C. S.; Miles, L. A.; Grove, T. L.; Booker, S. J.; Krebs, C.; Walsh, C. T.; Bollinger, J. M. *PNAS*. **2009**, *106*, 17723-17728.
10. Wong, S. D.; Srnec, M.; Matthews, M. L.; Liu, L. V.; Kwak, Y.; Park, K.; Bell, C. B.; Alp, E. E.; Zhao, J.; Yoda, Y.; Kitao, S.; Seto, M.; Krebs, C.; Bollinger, J. M.; Solomon, E. I. *Nature*. **2013**, *499*, 320-323.