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Generation of Metal Substituted Heme Oxygenase

Heme oxygenase (HO) is an enzyme with many newly discovered physiological roles including cellular signaling and antioxidant defense. Its primary function is to catalyze the decomposition of heme from blood that is trapped in tissue during an injury; this trapped heme manifests itself as a purple bruise due to color of heme. The decomposition catalyzed by HO proceeds through three O2-dependent oxygenation reactions, sequentially forming α-meso-hydroxyheme (purple), verdoheme (green) and billiverdin (yellow). The conversion to soluble biliverdin results in the release of the chelated Fe, and allows the decomposition products to be washed from the injury. We plan to study the reactivity of metal-substituted heme oxygenases, where the iron atom has been replaced by manganese or cobalt, in order to test mechanistic questions regarding the catalytic activity.

In the lab, inducement of the native HO reactivity can be generated with simple reductants or oxidants; e.g., the reaction of native HO with sodium ascorbate and oxygen yields biliverdin while the reaction with hydrogen peroxide stops at the intermediate verdoheme form. Initial work in our lab has demonstrated catalytic turnover of manganese-substituted HO in the presence of oxygen and sodium ascorbate, as well with hydrogen peroxide. But unlike the native iron enzyme, the manganese substituted form stops at the analogous verdoheme product. To the best of our knowledge, there is no precedence of catalytic turnover of HO with metal-substituted hemes. We hope to understand this change in actiivity, and to test it with an oxygen analogue, HNO, which is under study in our lab.

In the proposed work, an undergraduate researcher in our group, Azaan Ramani, will express HO using an existing protocol, reconstitute the enzyme with different metal-substituted hemes, and characterize the resulting modified enzymes. He will then assist graduate student Tara Clover in the use of these metal-substituted HO samples to determine the metal-dependency of the three sequential oxygenation reactions of HO, as well as the possibility of substitution of HNO for O2 in these reactions. We expect to show tangible results from this work- peer-reviewed journal publications and regional or national scientific conferences.