Title - A pH Dependant Switch in DHP Oxidation Mechanism

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Abstract: Heme-containing enzymes capable of oxidizing harmful aromatic substrates have recently become of significant interest, particularly in the pharmaceutical and agrochemical industries where the use of fluorinated compounds has increased in recent years. These compounds have a variety of functional uses in these industries, particularly as components of pesticides and drugs. There are, however, some problems with the use of these fluorinated compounds, as their metabolic waste products can produce potentially toxic or carcinogenic compounds[1, 2]. In many cases, oxidation of these compounds alleviates their harmful properties, thus the use of heme peroxidase enzymes in remedial or waste treatment strategies has received considerable attention. An issue this brings to light is that the effectiveness of peroxidase enzymes is often limited by the mechanism through which they operate, as they oxidize substrates by adding an oxygen atom derived from water through a radical mechanism. Free radicals are inherently unpredictable in the chemistry they perform, which makes control of these reactions very difficult. Enzymes that oxidize these same substrates through a peroxygenase mechanism have the potential to be much more specific and effective since they operate through a direct oxygen transfer mechanism deriving the oxygen atom from hydrogen peroxide[3]. Recently the multifunctional enzyme dehaloperoxidase (DHP) has become of interest with regards to substrate oxidation reactions. DHP is a hemoglobin-like protein found in the benthic marine worm *Amphitrite ornata*. In addition to its function as an oxygen carrier, DHP has also been shown to possess both peroxidase and peroxygenase functions[4]. *A. ornata* lives in an environment highly contaminated by toxic metabolites secreted by other organisms, however DHP allows *A. ornata* to survive by oxidizing those toxic compounds. We have

recently shown that the formation of certain products by DHP switches in a pH-dependant manner when using 4-fluorophenol as a substrate. We believe that this represents a pH dependant change in mechanism. At pH 5, 4-fluorocatechol is produced, however between pH 5 and pH 7.5, the formation of 4-fluorocatechol gradually decreases and the formation of an unknown product gradually increases. A 2016 study used 4-nitrophenol as a substrate and produced 4-nitrocatechol as a product. The study used isotope labelling to show that the oxygen atom used to oxidize the substrate was derived exclusively from H_2O_2, consistent with a peroxygenase rather than a peroxidase mechanism[5]. This suggests that the catechol product we are producing at pH 5 is also the result of a monooxygenase reaction, while the second product produced at a higher pH is likely the product of a radical mediated peroxidase reaction. It seems likely that pH is a critical control of the switch between the two oxidation mechanisms. The next steps in our study will focus on characterizing the second product produced by DHP. We will then proceed to perform isotope labeling studies, using O^{18} water and hydrogen peroxide, performing mass spectrometry on the reaction products to identify the origin of the oxygen atom used to oxidize the substrate to definitively identify the type of oxidation reaction occurring at various pH values.

Christian Worldview Integration: As researchers we are constantly seeking to better understand God's creation and the intricacies of the world we live in. Enzyme catalysis is one of the most vital functions that organisms perform, and without enzymes life simply could not function. As such, a better understanding of enzymes and the reactions they catalyze gives us just a glimpse into the systems God has implemented to allow life to function as we know it. Further, God calls us to be good stewards of the world he has placed us in. This includes protecting the environment and being intentional about how we utilize the resources we've been entrusted with. For example advances in the agrochemical industry have allowed us to greatly increase crop yields and feed many more people for far less cost and effort, however with the use of this type of technology it is also important for us to understand how the

compounds we are producing are metabolized by humans, animals, and other plants. Likewise in the pharmaceutical industry, advances in drug development have greatly improved health and quality of life for millions of people, however with these advancements, as well as the future development of new remediation techniques, we must understand how the compounds we use are degraded and metabolized, and what the effects of various compounds are beyond their desired function. Our work in enzyme catalysis and the oxidation of fluorinated compounds is one step towards a better understanding of these processes, and will hopefully be beneficial for future developments in various industries.

Sources

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