

Dehaloperoxidase (DHP) is an enzyme originating from the terebellid polychaete *Amphitrite ornata* that, when reacted with fluorophenol, has been found by previous studies to have a pH-dependent reaction mechanism. In peroxidase reactions, the oxygen found in the product originates from the surrounding water in the aqueous solution. As part of the intermediate in this reaction, a phenoxy radical is generated which can rearrange or disproportionate. Ultimately, a cation is formed, and water (a nucleophile) quenches it. At a pH of 7, a “typical” peroxidase reaction occurs generating the same cation intermediate which is then attacked by water and the subsequent free radicals form dimers or are quenched on more substrate. However, at a lower pH the reaction between DHP and fluorophenol generates a catechol, a product uncharacteristic of any peroxidase reaction. The hypothesis of this research project is that the mechanism of the DHP reaction changes from a peroxidase mechanism at a high pH to a peroxygenase mechanism at a low pH. To determine DHP’s pH-dependent mechanism, DHP and fluorophenol were reacted and analyzed using High Performance Liquid Chromatography (HPLC) at both pH 7 and pH 5. As a result of these experiments, the catechol was observed to be formed only at a pH of 5. In addition, the formation of the catechol was greatly enhanced by ascorbic acid. Because peroxygenase chemistry is predictable and specific, this method could be used to screen for peroxygenase reaction mechanisms. For example, peroxidases are abundant in nature, but those capable of peroxygenase chemistry are rare. Screening multiple peroxidases by looking for the catechol product of this reaction would be a simple effective way to identify novel peroxidases with this property.