Synthesis and Reactivity of Alkene Dioxygenase Model Complexes

Submitted by

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Abstract

Carotenoids are a family of molecules that are categorized by their alternating carbon-carbon double bonds, and are responsible for a variety of important processes in all forms of life. Carotenoid Cleavage Dioxygenases (CCDs) are iron-containing enzymes capable of converting carotenoids to biologically important molecules, which are critical for biological functions in plants and animals. CCDs cause specific carbon-carbon double bonds in carotenoids to break. In this study, the investigation of CCDs and similar structured enzymes using small molecule model compounds will help to determine the relationship between structure and reactivity. This relationship can be tested by using iron-containing compounds for enzyme modeling with continual structure adjustments and reacting said compounds with relevant organic molecules. This will result in further knowledge of enzymes and the creation of compounds that can be used as catalysts to assist in the determination of reaction mechanisms. This information will advance understanding of reactions previously proposed by experimental and theoretical investigators in this field.

Current Research

In biological systems, enzymes containing a metal cofactor are an important subclass of proteins. These “metalloenzymes" are capable of producing many biological transformations. Carotenoids, a type of functional group composed of alternating carbon-carbon double bonds (alkenes), are precursors for important biological activities in both plants and animals which are processed by a certain iron-containing enzyme categorized as Carotenoid Cleavage Dioxygenases (CCDs).[1] CCDs are enzymes that convert carotenoids to biologically-active molecules.[2,3] In plants, the reactions of cartinoid-derived products are responsible for important biological activities, including photosynthesis and cell membrane flexibility.[4] In animals, these products are needed for sight, fetal development, and cellular stability.[5,6]

The iron is bonded to CCDs through four histidine groups. To study how these enzymes carry out their function, small molecular model complexes for the active site of CCDs were made. After making these compounds, the reaction with oxygen and substrates were studied to determine if the hypothesized reaction pathways, the pathway that enzymes modeled, were likely. The compounds coordinated to the center iron atom varied in bulk to experiment with controlling the coordination number (number of groups bonded to iron). A five-coordinate imidazole complex, $[Fe(1,2\text{-}Me_2Im)_5](OTf)_2$ (**1**), is formed when 6 equivalents of 1,2-dimethylimidazole is added to $Fe(OTf)_2{\cdot}2CH_3CN$ in dichloromethane. Similarly, a four-coordinate complex, $([Fe(1\text{-}Me\text{-}2\text{-}^{i}PrIm)_4](OTf)_2$ (**2**) is made when 6 equivalents of 1-methyl-2-isopropylimidazole is combined with $Fe(OTf)_2{\cdot}2CH_3CN$ in dichloromethane. Steric bulk is a factor in determining imidazole group coordination to a central iron atom.

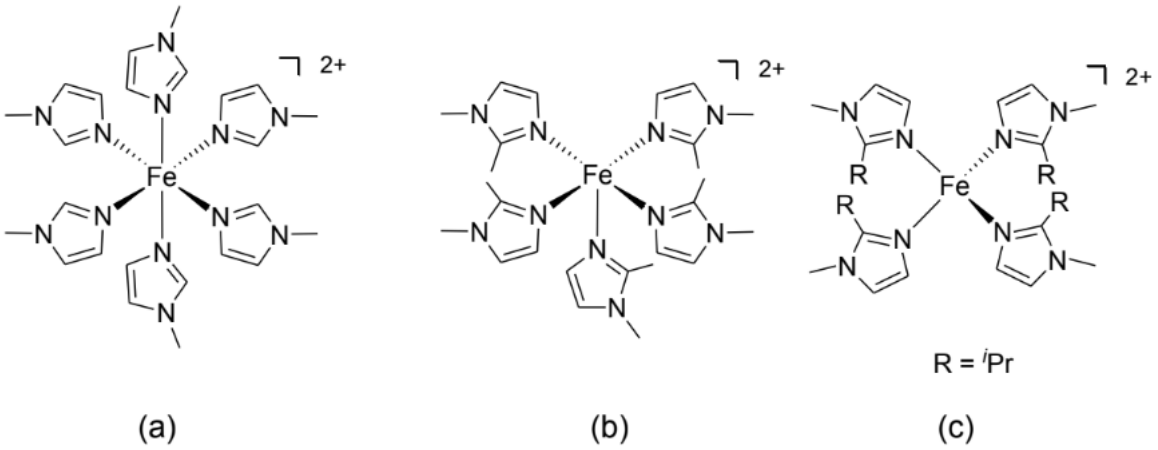

Figure 1. Structures of (a) $[Fe(TrIm)_4(OTf)_2]$, (b) $[Fe(1,2\text{-}Me_2Im)_5]\text{-}(OTf)_2$ (**1**), and (c) $[Fe(1\text{-}Me\text{-}2\text{-}^{i}PrIm)_4](OTf)_2$ (**2**).

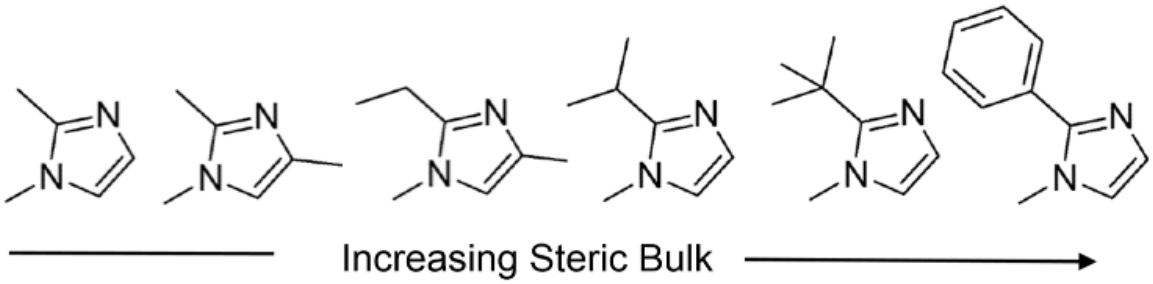

Figure 2. Examples of Substituted Imidazole Ligands with Increasing Steric Bulk.

By making use of various research-level laboratory equipment and techniques that the Chavez research team has established, these tasks were accomplished in a systematic manner. These experiments will expand on previous work done with related model complexes.

The continued investigation into the creation and experimentation of synthetic model complexes will further expand the knowledge of the Chavez lab group. So far, the group has progressed in creating the model enzyme complexes and studying the oxygenated intermediates

using different spectroscopy methods.[7] Through the use of NMR, IR, UV-Vis, X-Ray Crystallography, etc., the mechanisms of reactivity in these enzymes can be proposed, as proven by reaction mechanisms written previously by the group.[8]

Aims and Objectives

Introduction

The overall goal of this project is to create accurate model compounds that react in a similar way to CCD enzymes and categorize each step of the reactions. This was accomplished over time by planned experiments and analyzing the results carefully through spectroscopic methods such as IR, GC-MS, and NMR.

Aims

1. To synthesize model compounds of histidine groups that resemble the active sites of the CCD enzymes, involving iron and varying bulky imidazole groups.

2. To characterize the model compounds that were synthesized.

3. To react model complexes with added 1-bromo-4-(1-methoxyprop-1-en-2-yl)benzene (alkene) and oxygen.

4. To propose a reaction mechanism.

Objectives

1. Synthesizing model compounds will create a representation of the enzymes found in nature, therefore with these compounds, reactions can be pursued that are representations of real reactions catalyzed by CCDs. A standardized iron compound ($Fe(OTf)_2 \cdot 2CH_3CN$) will be reacted with imidazole compounds (models for Histidine). The imidazoles, otherwise known as ligands, will vary in the bulk of the substituents. These newly synthesized compounds will act as models for the active site of CCDs.

2. Formed model complexes will be studied using spectroscopy methods in order to verify expected structure and evaluate properties. This will ensure the correct complexes were made and give additional information to future researchers.

3. By reacting the compounds with oxygen, a comparison was made with the reactions found in CCDs in nature. Alkene substrates were added to the model complex to evaluate the yield of alkene cleavage, a representation of CCD cleavage in biological reactions. Then, reactions with oxygen were completed. The reaction intermediates (unstable compounds seen only during the reaction) are essential for proposing a reaction mechanism scheme and give information about what compounds can be isolated from the reaction. The intermediates were studied and categorized using spectroscopy methods. These reactions were essential in proposing a reaction mechanism.

4. Reaction mechanism will be proposed after the previously listed experimentation is completed. Potentially, this could guide future researchers to investigate similarly structured CCD enzyme models utilizing a different metal element other than iron.

Methodology

Methods and Materials

To create the models for the CCD enzyme active site, 4-Histidine model compounds were reacted with bulky nitrogen-containing molecules. The model compounds were then bound with iron. Different metals have been used by our group in the past, but this project specifically deals with iron-containing compounds. This process was done in a glove-box since these are air-sensitive chemicals and exposure to the open air would defeat the purpose of reacting them with oxygen separately. The iron compound needed in all reactions, has already been synthesized by the Chavez lab group. The bulky imidazole compounds needed for the reactions have been synthesized by the lab as well. The imidazole compounds made are 2-isopropyl-1-methylimidazole and 1,2-dimethylimidazole. Crystalline samples were prepared and elemental analysis was conducted.

After the model compounds were synthesized, the next step was to react these compounds with oxygen in the presence of a substrate compound. This was done by bubbling oxygen from an oxygen tank into the furnished compounds dissolved in solvent (usually dichloromethane) at a variety of different temperatures. The ratio of model complex to alkene was adjusted to investigate turnover rate of the model complex. Possible reaction intermediates were hypothesized. Notes were made about the colors observed for each type of reacted compound.

The goal of this project was to propose a reaction mechanism. Therefore, each step of the reaction was studied carefully. Spectroscopic methods such as IR, GC-MS, and NMR were used to check the reactants for purity, determine products, and eventually determine the relationship between these enzymes and their substrates. While evaluating the reactions between substrates and alkene, relative amounts of an internal standard (anthracene or naphthalene) were added to the reaction mixture before the analyses were performed. The reaction products were compared in calculations to determine reaction yield. These spectroscopy methods will categorize these reactions as catalytic or stoichiometric.

$Fe(OTf)_2 \cdot 2CH_3CN$ was made according to a previous literature procedure.[9] Methyl groups were added to 2-Isopropylimidazole (Aldrich) following a literature procedure.[10,11] 1,2-dimethylimidazole (Alpha Aesar) was used as received. 1-bromo-4-(1-methoxyprop-1-en-2-yl)benzene was made according to literature procedure.[12] Oxygen (99.6%) was obtained from Praxair and Oxygen-18 was purchased from ICON-Isotopes, 98.2 atom percentage. $CDCl_3$ was obtained from Cambridge Isotopes Laboratories, Inc. All other reagents were purchased from commercial sources and used as received. Pure dry solvents were acquired using an Innovative Technologies, Inc. Solvent Purification System. Air-sensitive manipulations were performed using an Innovative Technologies, Inc. nitrogen-filled glovebox or by using standard Schlenk-line techniques. Elemental analysis was performed on powder crystalline samples that were placed under vacuum and sealed in glass ampules prior to submission to Atlantic Microlabs, Inc., Norcross, GA.

1-Me-2-iPrIm Synthesis. To 100 mL of tetrahydrofuran (THF), NaH (.460 g) was added slowly. The mixture was then refluxed for 1.5 hours then cooled to room temperature. CH_3I (2.77 g) was added. Product in THF was formed, and separated from the formed solid iodine via rotary evaporator. To collect dry 1-Me-2-iPrIm, a distillation method was used. Yield: 1.89 g (85%).

[Fe(1,2-Me$_2$Im)$_5$](OTf)$_2$ Synthesis. To a stirring solution of $Fe(OTf)_2 \cdot 2CH_3CN$ (221 mg, 0.51 mmol) in 2 mL of DCM, 1,2-dimethylimidazole (250 mg, 2.6 mmol) dissolved in 2 mL of DCM was added. The solution was filtered and diethyl ether was added after 1 hour of stirring. The solution was then placed at -30°C. After 24 hours, colorless blocks were deposited. The crystals were collected and washed with ether. Yield: 370 mg (87%). Anal. Calcd for $C_{27}H_{40}F_6FeN_{10}O_6S_2$ (**1**): C, 38.85; H, 4.83; N, 16.78. Found: C, 38.82; H, 4.88; N, 16.73. FTIR (cm^{-1}): 3139 (w), 2959 (w), 1505 (m), 1418 (m), 1258 (s), 1225 (m), 1160 (m), 1138 (m), 1093 (m), 1002 (m), 945 (m), 773 (m), 735 (s), 634 (m), 572 (s), 516 (m), 441(m) cm^{-1}. UV-vis: λ [(ε, M^{-1} cm^{-1}) in CH_2Cl_2] = 287 (405, sh). Magnetic measurements, $x_M T$ (polycryst, 298 K): 3.60 cm^3 mol^{-1} K.

[Fe(1-Me-2-iPrIm)$_4$](OTf)$_2$ Synthesis. To a stirring solution of $Fe(OTf)_2 \cdot 2CH_3CN$ (220 mg, 0.50 mmol) in 2 mL DCM, 2-Isopropyl-1-methylimidazole (255 mg, 2.1 mmol) dissolved in 2 mL DCM was added. After 1 hour of stirring, the solution was filtered and diethyl ether was added. The solution was then placed at -30ºC. After 24 hours, colorless needle-like crystals were deposited. The crystals were collected and washed with ether. Yield: 379 mg (89%). Anal. Calcd for $C_{30}H_{48}F_6FeN_8O_6S_2$ (**2**): C, 42.35; H, 5.69; N, 13.17. Found: C, 42.31; H, 5.62; N, 13.07. FTIR

(cm^{-1}): 3133 (w), 2982 (w), 1550 (w), 1487 (m), 1258 (m), 1221 (s), 1141 (m), 1072 (m), 1026 (s), 958 (m), 785 (m), 735 (s), 697 (m), 640 (m), 571 (m), 516 (s), 421 (m) cm^{-1}. UV-vis: λ [(ε, M^{-1} cm^{-1}) in CH_2Cl_2] = 280 (578, sh), 373 (100, sh). Magnetic measurements, $x_M T$ (polycryst, 298 K): 3.28 cm^3 mol^{-1} K.

1-bromo-4-(1-methoxyprop-1-en-2-yl)benzene Synthesis. To a stirring solution of [methoxymethyl] triphenyl-phosphonium chloride (43.5 g, 76.3 mmol) and THF, potassium t-butoxide (8.56 g, 76.3 mmol) was added via solid addition funnel over ice. 4'-bromoacetophenone (11.7 g, 50.9 mmol) was then added via liquid addition funnel gradually. A creamy orange color formed and was left overnight to stir and warm to room temperature.

Physical Characterization. ^{1}H NMR spectra were recorded at 25°C on a Bruker Avance II 400 MHz instrument and sample peaks in [D]chloroform were referenced to TMS (tetramethylsilane). FT-IR spectra were measured on a Bruker ATR Alpha P spectrometer. Optical spectra were collected on a Cary 50 UV-vis spectrophotometer. GCMS experiments were performed on an HP 6890/5973 GCMS.

Reactivity Studies. The stoichiometric reactions were carried out in a fume hood as 22℃. 0.07 mmol of $[Fe(1,2\text{-}Me_2Im)_5](OTf)_2$ or $[Fe(1\text{-}Me\text{-}2\text{-}iPrIm)_4](OTf)_2$ and 1-bromo-4-(1-methoxyprop-1-en-2-yl)benzene (0.26 mmol) dissolved in 4 mL CH_2Cl_2 under nitrogen was added to a 25 mL Schlenk flask. The flask was sealed then sparged with oxygen (Airgas, Industrial grade) or oxygen-18 (ICON Isotopes, 98.2 atom percentage) for 1 minute. The

solution was stirred for 14 hours after stoppering. 4 mL of 2 M HCl was then added and the mixture was shaken. The organic layer separated from the aqueous layer, and the aqueous layer was extracted (3 times, each with 3 mL of CH_2Cl_2). The organic layers were combined and dried over anhydrous sodium sulfate. The solvent was then evaporated by vacuum.

Outcomes

Enzyme models were successfully synthesized, and product yield was determined for the following model compounds. The individual models were checked through elemental analysis to confirm the correct compound was made. Further characterization was completed through NMR and IR spectroscopy. The model compounds were then reacted with 1-bromo-4-(1-methoxyprop-1-en-2-yl)benzene and the ratio of model complex to alkene was adjusted to investigate turnover rate. Other compounds, such as $Fe(OTf)_2 \cdot 2MeCN$ were also synthesized and reacted with alkene and then oxidized. Excess alkene in dichloromethane under nitrogen was used to ensure factors other than amount of substrate influenced product yield.

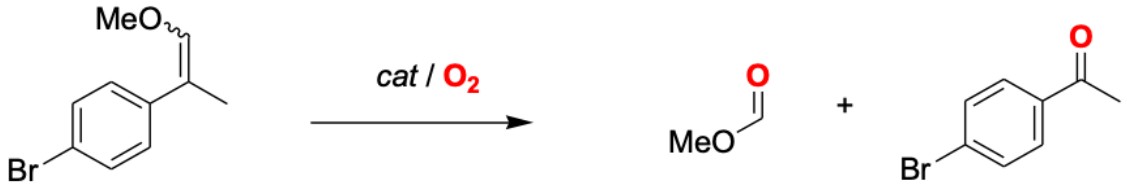

Figure 3. Model Reaction with 1-bromo-4-(1-methoxyprop-1-en-2-yl)benzene.

Compound	Ratio of Metal to Alkene	Number of Hours Left Stirring	Oxidant	Product Yield
1	1:10	14	O_2	29%
1	1:10	24	O_2	27%
2	1:10	14	O_2	50%
2	1:10	24	O_2	48%
2	1:10	14	$^{18}O_2$	45%
$Fe(OTf)_2 \cdot 2MeCN$	1:10	24	O_2	5%

Table 1. Reactions with 1-bromo-4-(1-methoxyprop-1-en-2-yl)benzene.

After the reaction, the resulting contents were analyzed by GCMS. When bubbling in $^{18}O_2$, O-18 was observed in methylformate and 1-(4-bromophenyl)ethanone was produced. As seen in Table 1, using compound **2** in the reaction produces higher product yield than compound **1**, when both are stirred for 14 hours. This result is likely due to the higher Lewis acidity of **2** compared to **1**.

Positioning of the O-18 once incorporated into both the ketone and aldehyde products indicates the production of a dioxetane (a four-sided compound with two oxygen molecules connected) intermediate in the reaction. The product solution changed to a dark brown color when exposed to oxygen, which also supports this intermediate. In the following step, the ester product, O-18 is observed in both places oxygen can be present in the molecule. This may be due

to the isolation process after the product is produced. The proposed reaction featuring the dioxetane intermediate is shown in Figure 4.

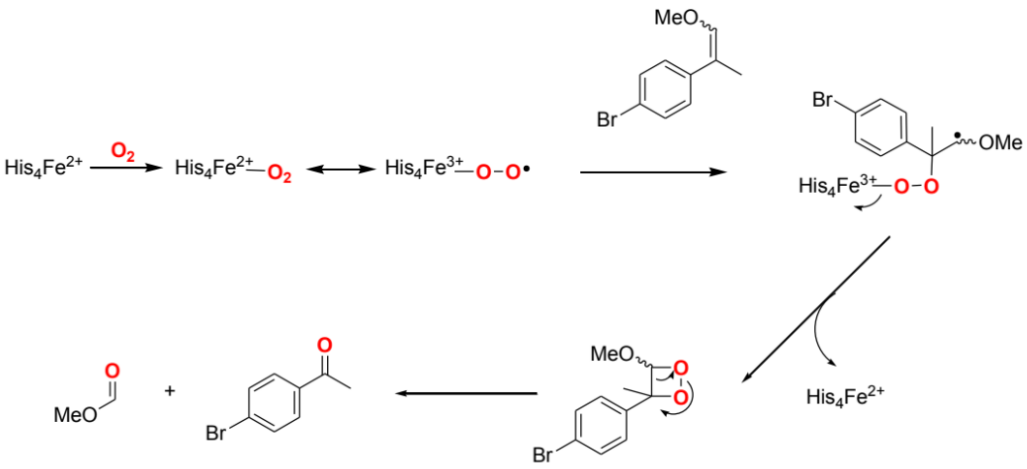

Figure 4. Proposed mechanism of 1-bromo-4-(1-methoxyprop-1-en-2-yl)benzene cleavage for model studies.

In addition, density functional theory calculations (DFT) completed by Dr. Chavez's collaborators, Priya Singh and Dr. Timothy A. Jackson (University of Kansas), indicate the pathway proposed in Figure 5. DFT measures the energy changes of intermediates in the reaction pathway. In general, if the energy needed continually decreases, then that outcome of the reaction is favored. This is the trend seen in the calculations completed, which further gives evidence indicating the dioxetane rather than the epoxide mechanism, as noted in Figure 5.

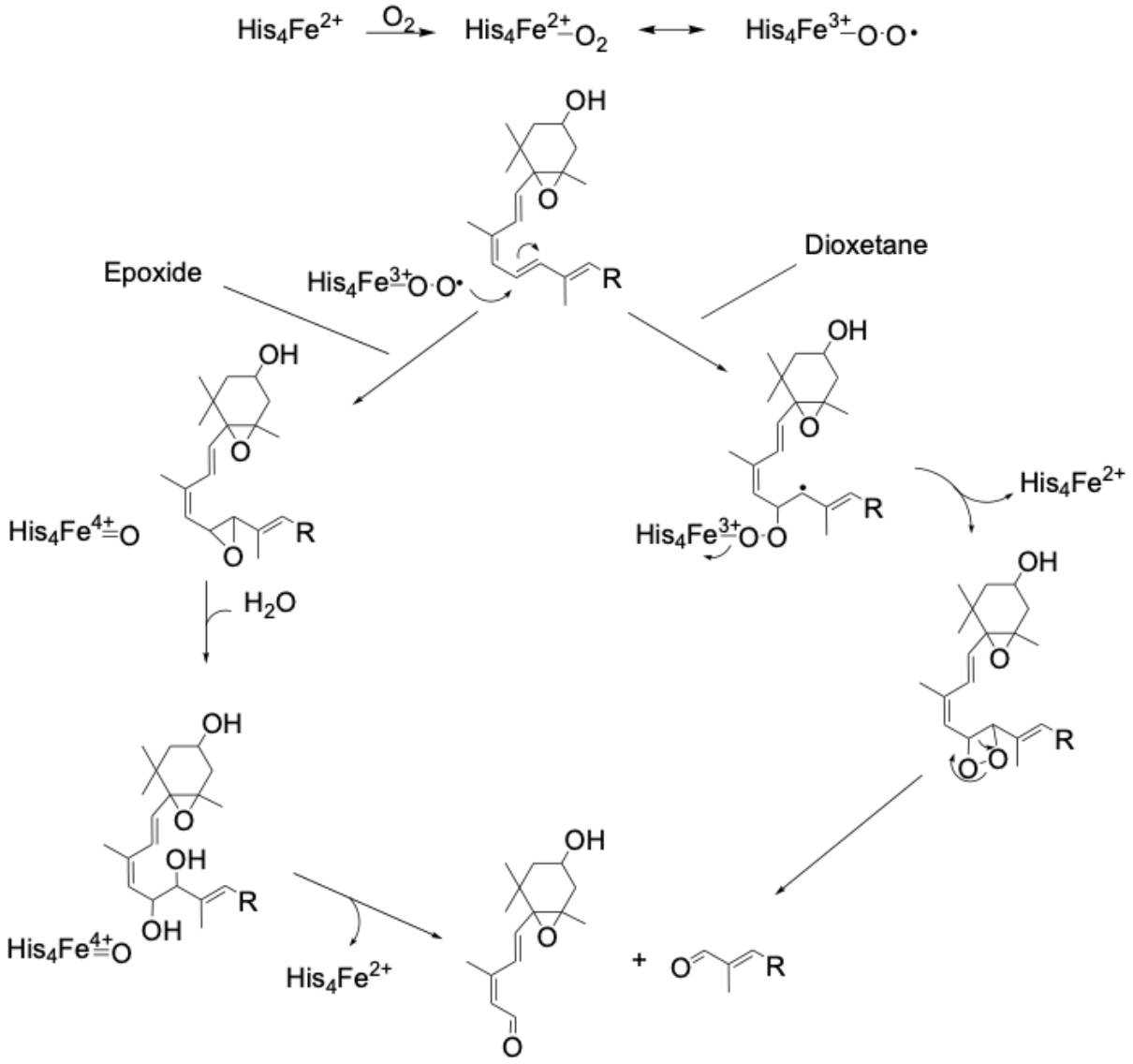

Figure 5. Proposed dioxygenase mechanism.

These studies demonstrate that a model complex for alkene dioxygenase is capable of performing the enzymatic reaction in a stoichiometric fashion. Furthermore, reaction intermediates and results with O-18 for compound **2** support the dioxetane mechanism for the enzyme and is a model example of oxidative olefin cleavage reaction. DFT studies support the

proposed mechanism which is downhill. Turnovers are not observed which is attributed to a possible rearrangement of the final complex. Results demonstrate that the enzymatic reaction involves a dioxetane intermediate to produce cleavage products.

Biographical Note

I am an undergraduate student completing my Bachelor's of Science in Biochemistry and a minor in Spanish. I am graduating in the Winter semester of 2023. I will be pursuing a Doctor of Pharmacy degree at the University of Kansas in fall of 2023. I have a great interest in applied science to biological organisms, hence the biochemistry degree and my focus in bioinorganic chemistry for my thesis project. The experience I gained while working on my thesis will be more valuable than any class or job that I could have during my undergraduate degree. The knowledge I have gained through working in the laboratory and presenting my research has guided my choices in classes and my future career.

I would like to thank Dr. Ferman Chavez for his support and guidance throughout this project. I would also like to acknowledge all the collaborators on the Chavez lab group project which guided the focus and outcomes of my contributions including; Dr. Atanu Banerjee of Charotar University of Science and Technology (CHARUSAT), Selin Senturk of Oakland University, Dr. William W. Brennessel of the University of Rochester, Dr. Andreas Omlor and Dr. Volker Schünemann of University of Kaiserslautern, Priya Singh and Dr. Timothy A. Jackson of The University of Kansas. Lastly, I acknowledge the undergraduate OU Provost award.

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