

Are Vanadium Intermediates Suitable Mimics in Non-Heme Iron Enzymes? An Electronic Structure Analysis

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ABSTRACT: Vanadyl intermediates are frequently used as mimics for the fleeting Fe(IV)=O intermediate in non-heme iron enzymes that catalyze C–H activation. Using density functional theory and correlated wavefunction theory, we investigate the degree to which vanadium mimic electronic structure is comparable to catalytic iron intermediates. Our calculations reveal crucial structural and energetic differences between vanadyl and ferryl intermediates primarily due to differences in ground spin states of low spin and high spin, respectively. This difference in spin state leads to differences in energetics for accessing isomers that confer activity in non-heme hydroxylase and halogenase enzymes. While interconversion between monodentate and bidentate succinate isomers of the key metal-oxo/hydroxo intermediates is energetically favorable for Fe, it is strongly unfavorable in V mimics. Additionally, isomerization of a terminal metal-oxo between an axial and equatorial position is energetically unfavorable for Fe but favorable for V. Electronic structure analyses to quantify differences in binding strength of Fe and V intermediates to α -ketoglutarate and succinate cosubstrates reveal that both cosubstrates bind more strongly to V than Fe. Our work highlights the limits of using low- or intermediate-spin V-based intermediates as mimics in studies of fleeting high-spin Fe intermediates in non-heme iron enzymes.

1. Introduction.

Selective C–H activation is essential¹ for the synthesis of bioactive molecules²⁻⁴ and natural products⁵⁻¹³, medicinal chemistry^{3, 14-17}, the materials industry¹⁸⁻²⁰, and agricultural industry²¹⁻²³. Synthetic routes to activate unreactive C–H bonds require harsh conditions²⁴ and usually show poor selectivity²⁵ owing to the high C–H bond dissociation energy and its inertness due to low polarity²⁶. Biological systems, such as non-heme iron enzymes,²⁷⁻³⁴ carry out selective C–H activation of unreactive C–H bonds³⁵⁻⁴¹ efficiently at ambient conditions to catalyze a wide variety of reactions^{37, 42-44}. These enzymes^{37, 42-46} play a crucial role in numerous biosynthetic processes⁴⁷⁻⁵² such as primary and secondary metabolism in plants⁴², generation of clinically relevant natural products⁵³⁻⁵⁴, DNA repair⁵⁵⁻⁵⁸ and transcription⁵⁹⁻⁶¹.

The active site of the α -ketoglutarate (α KG)-dependent non-heme iron halogenases^{27-28, 30-34, 62} is similar to that of hydroxylases⁶²⁻⁶³ where chloride replaces the carboxylate of the canonical 2-His-1-carboxylate facial triad^{28, 64-65} bound to an Fe center. The Fe(IV)–oxo and Fe(III)–hydroxo intermediates formed during the catalytic cycle of these enzymes are highly reactive^{44, 66-70} which makes them hard to isolate and characterize by experimental techniques of crystallography⁷¹⁻⁷² or spectroscopy^{71, 73-76}. An experimental approach to structurally characterize these fleeting Fe(IV)–oxo and Fe(III)–hydroxo intermediates frequently invoked in C–H activation^{69, 77} is to replace them with vanadium intermediates that are more inert (Figure 1). A search of the protein data bank (PDB)⁷⁸ confirms that there are numerous crystal structures of the resting state intermediate of Fe(II)/ α KG enzymes^{28, 32} while an Fe(IV)–oxo intermediate with succinate and two His residues bound to Fe is absent (Figure 1). However, numerous crystal structures of V(IV)–oxo intermediates have been obtained for non-heme oxygenases^{77, 79-80} and hydroxylases⁸¹ (Figure 1). While these vanadyl intermediates are easier to characterize

experimentally, comparing the metal-ligand (M–L) bond distances between Fe/V and His residues reveals considerable differences. For example, the equatorial Fe–N_{His} distance is significantly longer (i.e., beyond the positional uncertainty in the crystal structure⁸²) than the V–N_{His} distance (by 0.15 Å) despite comparable axial M–N_{His} distances (M=Fe, V, Figure 1).

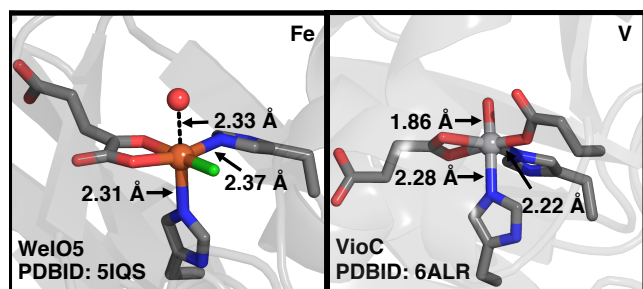


Figure 1. (Left) Active sites of non-heme iron halogenase, WelO5 (PDB ID: 5IQS) and (right) non-heme vanadium hydroxylase, VioC (PDB ID: 6ALR). The metal-ligand distances between Fe and water, Fe and His (both axial and equatorial), V and oxo, and V and His (both axial and equatorial) are indicated in the insets. Carbon, nitrogen, oxygen, chlorine, vanadium, and iron are shown in grey, blue, red, green, silver, and brown, respectively.

Prior studies suggested that one or both of the fleeting Fe(IV)–oxo and Fe(III)–hydroxo intermediates formed before and after hydrogen atom abstraction, respectively, isomerize to favor halogenation as opposed to hydroxylation^{83–87}. Isomerization of the active site in non-heme iron halogenases is expected to play an important role in substrate positioning and reaction selectivity^{32, 87}. Since it is hard to capture these fleeting iron intermediates experimentally, numerous computational studies^{83–84, 88–91} have been carried out to investigate the mechanism of selective halogenation by non-heme iron halogenases. While the crystal structures obtained with Fe(II)/ α KG suggest the formation of axial oxo^{28, 32, 92} in most cases, isomerization to equatorial oxo is possible^{40, 85}. Previous studies have alternately favored and disfavored isomerization as a necessary step in halogenation in a manner that depends on the enzyme and its substrate. For instance, while axial oxo appears favored in the carrier-dependent halogenase SyrB2^{28, 40, 83}, equatorial oxo is suggested in others such as the carrier-free WelO5, and the need for

isomerization has been less clear in the carrier-free BesD halogenase.^{32, 92-93} Although numerous enzymes have been characterized with vanadium centers, no computational studies have assessed the preference for isomerization with V-based mimics.

Mid-row iron-containing catalysts are well-known to exhibit spin-state dependent reactivity.^{74, 94-103} Non-heme iron halogenases and hydroxylases are believed to react on the high-spin (HS) ground state^{67-68, 93, 104}. In comparison to the electronic structure of mid-row Fe^{68, 83-85, 89, 105}, the earlier V¹⁰⁶ differs in electron configuration, resulting in different accessible spin states. While d^4 ferryl intermediates can access low-spin (LS) singlet, intermediate-spin (IS) triplet, and HS quintet states⁶⁵⁻⁶⁶ and are expected to prefer HS quintet states, d^1 vanadyl intermediates can only access an LS doublet state^{69, 77}. Additionally, it is unknown whether the spin is conserved in V(IV)–oxo and V(III)–hydroxo intermediates formed along the catalytic cycle.

In this work, we demonstrate limitations of using vanadyl mimics for the fleeting ferryl intermediates in C–H activation. We use density functional theory (DFT) and highly accurate correlated wavefunction theory (WFT) to evaluate both the most stable isomers of Fe and V intermediates as well as their isomerization energy landscapes. We show that the most stable isomers for the metal-oxo intermediate and the energetic differences between monodentate and bidentate succinate isomers differ significantly between ferryl and vanadyl intermediates. While the Fe(IV)–oxo isomerization reaction coordinate exhibits a high energy barrier, the corresponding isomerization is barrierless for vanadyl intermediate. These differences, which can be attributed to the difference in spin and electron configuration of the metal also lead to differences in binding strength of co-substrates such as succinate and α KG. Taken together, our

studies suggest caution in literal interpretation of vanadyl structures as faithful mimics of ferryl intermediates in mechanistic enzymology.

2. Reaction Mechanism.

Several studies have led to the proposal of a mechanism and likely intermediates^{28, 42, 44, 62, 107} in the catalytic cycle for non-heme iron halogenases. Starting from the resting state (**1**), the active site consists of an Fe(II) coordinated to two His ligands, a chloride ligand, a bidentate α KG ligand, and a water molecule which is loosely bound to the metal, producing a 6-coordinate active site (Figure 2). Upon the entry of substrate to the binding pocket, the water molecule is displaced (**2**), and molecular oxygen (**3**) binds to the active site (Figure 2). Once bound, molecular oxygen attacks the carbonyl carbon of α KG, leading oxidative decarboxylation of α KG and O–O bond cleavage (Figure 2). This step results in the release of a carbon dioxide molecule and an active site consisting of a terminal Fe(IV)–oxo intermediate coordinated to a succinate (**4**) co-substrate (Figure 2). The highly reactive Fe(IV)–oxo intermediate abstracts a hydrogen atom from the substrate, forming a radical substrate and an Fe(III)–OH intermediate (**5**) (Figure 2). This step is followed by rebound halogenation of the substrate radical by the chloride ligand to return of the active site to its resting state (**1**).

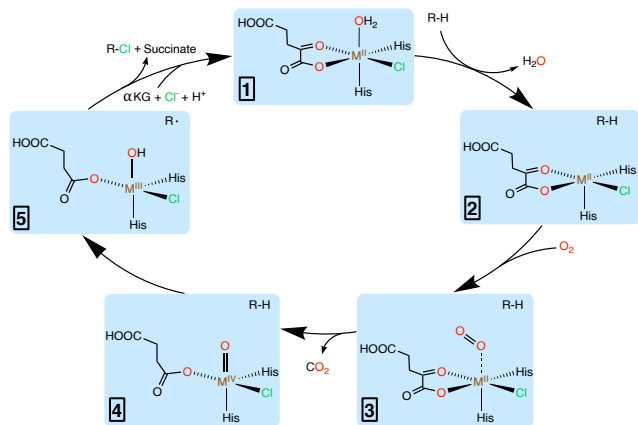


Figure 2. Proposed reaction mechanism of non-heme halogenases (clockwise, top to bottom):

the intermediates with water loosely bound to metal (**1**), water displaced from the active site when the substrate, R-H, enters the active site pocket (**2**), O₂ entering the active site (**3**), oxo and succinate (**4**), and hydroxo bound to metal (**5**) are shown. The metal, M = V, Fe, is shown in brown and the oxidation state for each intermediate is specified.

While this mechanism is largely agreed upon, the orientation of intermediates in the active site throughout the catalytic cycle as well as the degree of coordination by the α KG or succinate cosubstrate has been debated^{85, 89, 108-109}. For instance, in intermediates (**1**) or (**3**), the respective H₂O or O₂ moieties can be present in the axial or equatorial positions, giving rise to axial or equatorial configurational isomers (Supporting Information Figure S1). For the intermediates in the catalytic cycle after oxidative decarboxylation, succinate can alternately bind the metal in a monodentate or bidentate fashion. As a result, intermediates (**4**) and (**5**) have six isomers we compare since the succinate can be monodentate or bidentate combined with consideration of equatorial or axial oxo or hydroxo as well as the possibility that chloride is axial or equatorial (Supporting Information Figure S1).

3. Results and Discussion.

3.1. Spin State Confers Differences of Fe Intermediates from their V-based Mimics.

An experimental approach to structurally characterize the fleeting Fe intermediates frequently invoked in C–H activation^{69, 77} is to replace them with V-based mimics that are more inert. However, differences in electron configurations of Fe and V (e.g., d^4 Fe(IV) vs d^1 V(IV)) mean that intermediates will favor different spin states and have distinct electronic properties (Supporting Information Table S1). This difference could be expected to alter which isomers are predicted to be the most favorable between V and Fe intermediates. To understand the effect of spin state on geometries, energies, and isomer stabilities of intermediates, we study active site

isomers for both Fe and V in their corresponding LS, IS, and HS (for Fe only) states (Supporting Information Table S1, Text S1, and Figure S2). As could be expected from past experimental and computational studies^{44, 68, 110}, all Fe intermediates (i.e., whether Fe(II)–H₂O or Fe(IV)=O) prefer an HS ground state. On the contrary, the preference for LS vs IS states alternates in V intermediates, i.e., with IS for V(II)–H₂O and V(III)–OH, and LS for V(III)–O₂ and V(IV)=O intermediates (Supporting Information Table S2).

While V has a comparable covalent radius to HS Fe (i.e., 1.53 vs 1.52 Å), the preference for LS and IS states in V intermediates can be expected to lead to shorter M–L bond lengths with the reacting moiety. Indeed, the favored (i.e., LS or IS) V intermediates have M–L bond lengths with the reacting moiety that are always shorter (by 0.11 Å on average) than the HS Fe intermediates (Supporting Information Table S3). In contrast, V–Cl bonds are slightly longer than Fe–Cl bonds (by 0.03–0.08 Å), suggesting that the V–Cl bond is slightly weaker in all four intermediates (Supporting Information Tables S3–S4). While both M–N_{His} (N of His) bonds (M = Fe, V) are comparable for most isomers, the V–N_{His} or V–O_{suc} (O of succinate) bond opposite V–oxo is much longer than the other V–N_{His} or V–O_{suc} bond by ca. 0.30 Å in V(IV)=O isomers (Supporting Information Tables S3–S4 and Figure S3). Since we carried out these optimizations in the gas phase on cluster models amenable to WFT modeling, we also considered whether inclusion of screening effects from the protein environment could influence these conclusions. We find that inclusion of environment effects (i.e., with implicit solvent models) alters most gas-phase geometries very little (< 0.10 Å) except for the monodentate succinate, equatorial Fe(IV)=O, axial Cl⁻ isomer where the solvent-phase Cl–Fe–N_{His} angle is larger by 16° (Supporting Information Table S5 and Figure S4).

Since vanadium is most frequently used to mimic the reactive Fe(IV)=O intermediate immediately prior to hydrogen atom transfer (HAT), relative isomer energetics for the M(IV)=O and M(III)-OH intermediates must be comparable in order for vanadium to be viewed as a faithful mimic. Since these intermediates form after oxidative decarboxylation of α KG, the carboxylate of the succinate cosubstrate can bind the metal in a bidentate or monodentate fashion. While we attempt to understand six potential isomers for both M(IV)=O and M(III)-OH intermediates (i.e., with different positions of the reacting moiety as well as monodentate vs bidentate succinate), some of the monodentate structures rapidly optimize into a bidentate structure or otherwise cannot be stabilized. Thus, we focus on the relative energetics of four M(IV)=O isomers and five M(III)-OH isomers (Supporting Information Text S2 and Figure S5). For Fe, the relative energy of monodentate isomers is only slightly (i.e., 3–8 kcal/mol) higher than the bidentate cases, indicating the possibility that bidentate to monodentate interconversion could be accessible (Figure 3 and Supporting Information Figure S1). In contrast, the monodentate isomers of V intermediates are in most cases much less favorable: e.g., for the axial M(IV)=O isomer (Fe: 3 kcal/mol vs V: 18 kcal/mol) (Figure 3).

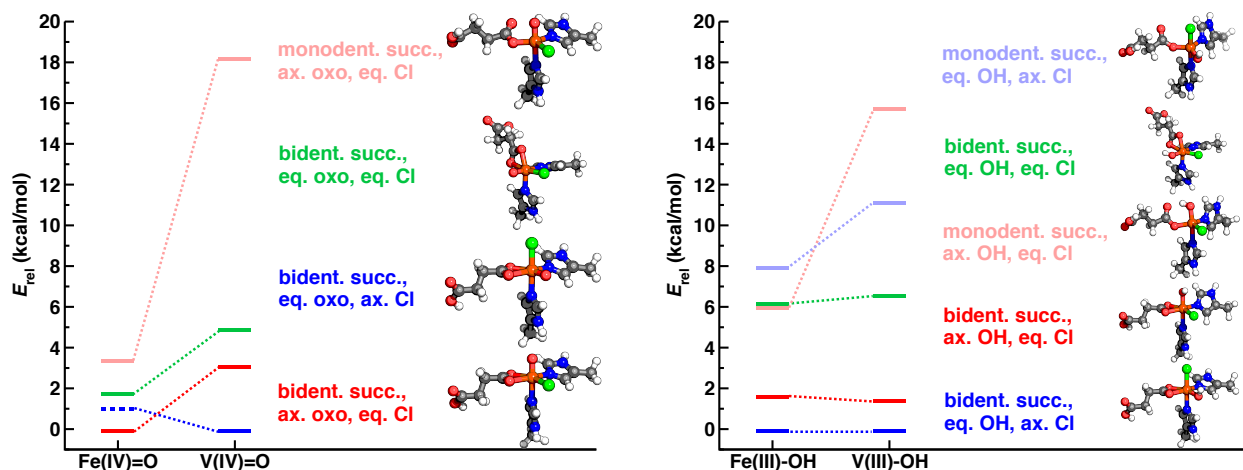


Figure 3. DLPNO/CBS energies (E_{rel}) of isomers of (left) M(IV)=O and (right) M(III)-OH (M = Fe, V) intermediates shown relative to the most stable isomer for each intermediate. Representative structures of Fe(IV)=O and Fe(III)-OH isomers are shown in the insets and

labeled. (Top to bottom, left) Isomers are axial (ax.) oxo, equatorial (eq.) Cl, monodentate (monodent.) succinate (succ.); equatorial oxo and Cl, bidentate (bident.) succinate; axial Cl, equatorial oxo, bidentate succinate; axial oxo, equatorial Cl, bidentate succinate. (Top to bottom, right) Isomers are axial Cl, equatorial OH, monodentate succinate; equatorial OH and Cl, bidentate succinate; axial OH, equatorial Cl, monodentate succinate; axial OH, equatorial Cl, bidentate succinate; axial Cl, equatorial OH, bidentate succinate. The dashed blue line corresponds to an estimated DLPNO/CBS energy. Hydrogen, carbon, nitrogen, oxygen, chlorine, and iron are shown in white, grey, blue, red, green, and brown, respectively.

In addition to differences in preference for succinate binding, there are differences in preferred configurational isomers for Fe vs V (Figure 3). While all bidentate isomers are fairly comparable in energy for Fe with the axial M(IV)=O isomer slightly favored, an equatorial M(IV)=O isomer with axial Cl⁻ is strongly preferred for V (Figure 3). Although differences are significant for the M(IV)=O moieties, energetics after the HAT step are more comparable between Fe and V for the M(III)-OH intermediate where the relative energetics of the three lowest energy bidentate isomers of Fe and V intermediates are comparable (Figure 3 and Supporting Information Figure S1).

To determine whether the differences between Fe(IV)=O and V(IV)=O intermediates are a result of the effect of the metal identity or the spin state, we next compare the corresponding isomers between similar spin state intermediates, i.e., IS triplet Fe(IV)=O and LS doublet V(IV)=O. Metal-oxo bond lengths of IS Fe(IV)=O and LS V(IV)=O are comparable (i.e., within < 0.05 Å), and all other M-L distances differ by < 0.20 Å except for specific isomers that have elongated V-N_{His} or V-O_{suc} bonds (Supporting Information Table S6). Further comparison of isomer energetics reveals that the isomer energetics for IS triplet Fe(IV)=O are comparable to those of LS doublet V(IV)=O. Both IS Fe(IV)=O and LS V(IV)=O have a strong preference for the bidentate succinate isomer which is stabilized by ca. 16 and 18 kcal/mol, respectively, over the monodentate succinate with an axial oxo (Supporting Information Figure S6). Among the

bidentate succinate isomers, both the active sites show a more moderate preference for equatorial oxo and axial Cl⁻ over the case of axial oxo (ca. 3 kcal/mol, Supporting Information Figure S6). Thus, the differences between HS Fe(IV)=O and LS V(IV)=O are largely a consequence of differences in their respective spin states (Supporting Information Text S3).

To understand the effects of metal on configurational isomer preference on other parts of the catalytic cycle, we also studied the two axial and equatorial isomers of M(II)-H₂O and M(III)-O₂ (M=Fe, V) intermediates with bidentate α KG (Supporting Information Figure S1). All M(II)-H₂O intermediates are characterized by comparably long (≥ 2.20 Å) M(II)-H₂O bond lengths indicative of a weak interaction consistent with observations from literature^{32, 111-112}, but the equatorial isomer is weakly favored (i.e., by 2.1 kcal/mol for Fe and 3.6 kcal/mol for V) (Supporting Information Figures S1 and S7, and Table S3). Both (i.e., Fe or V) M(III)-O₂ axial or equatorial isomers optimize to precursor structures in which O₂ attacks the carboxylate carbon of α KG, and they have comparable energetics (Supporting Information Figures S1 and S7 and Table S7).

Thus, analysis of the ground spin-state combined with the analysis of the geometries and preferred isomers indicates that there are significant differences between Fe and V active site isomers that are most apparent for the M(IV)=O intermediate where V is most essentially used as a mimic of Fe. This will strongly impact the interpretation of previous studies and should be taken into consideration while using V(IV)=O intermediates as mimics for fleeting Fe(IV)=O intermediates.

3.2. Metal-oxo Isomerization Energy Landscapes.

We next compared the isomerization energy landscapes of the M(IV)=O intermediate for both Fe and V in detail since isomerization of the fleeting Fe(IV)=O intermediate has been invoked in functionalization following C–H activation^{69, 77}. Our analysis of active site isomers (see Sec. 3.1) showed that the energetic difference between monodentate and bidentate succinate isomers for Fe(IV)=O is small (ca 3 kcal/mol). While the bidentate succinate isomer is slightly more stable than the monodentate succinate isomer, we study active site isomerization using monodentate isomers as it ensures the needed coordination flexibility in the active site to enable isomerization of the reacting moiety.

We study two isomerization reaction coordinates (RCs) for monodentate succinate isomers of both Fe(IV)–oxo and V(IV)–oxo intermediates. The first RC corresponds to isomerization between equatorial oxo and axial oxo in which Cl⁻ remains equatorial. This isomerization is captured by the change in N_{His}–M=O angle with the His trans to the axial oxo (Figure 4). For iron in this RC, we observe two minima: the global minimum for axial oxo and a local minimum for the equatorial oxo separated by a large barrier (ca. 8 kcal/mol) for isomerization from axial oxo to equatorial oxo isomer (Figure 4). We characterize the transition state (N_{His}–Fe=O angle: 127°) along this pathway by confirming a single imaginary frequency of ca. 132 cm⁻¹ along the RC. To isolate the effect of the metal center, we replaced Fe with V to obtain the equivalent V(IV)=O RC. In this case, a single minimum is observed that is significantly different than that for the Fe(IV)=O RC (Figure 4). While for Fe, the global minimum (N_{His}–Fe=O angle: 172°) is nearly the same as the axial oxo isomer, for V, the global minimum (N_{His}–V=O angle: 147°) shifts (Figure 4). As a result, this V-containing structure positions the oxo midway between the equatorial plane and the axial position (Figure 4). This preference is not an artifact of replacing Fe with V and constraining the geometry. If we instead

allowed all other degrees of freedom to relax in the V(IV)=O case as we did for Fe(IV)=O, the RC energy landscape is qualitatively unchanged (Supporting Information Figure S8). In some enzymes, it has been proposed^{32, 113} that equatorial oxo might be preferred for halogenation, but it would be challenging to corroborate this observation using vanadyl as a stand in for the ferryl intermediate. Our observations on the increased stability of the axial oxo indicate a high energy barrier for isomerization by non-heme Fe halogenases which is not accurately captured by V mimics.

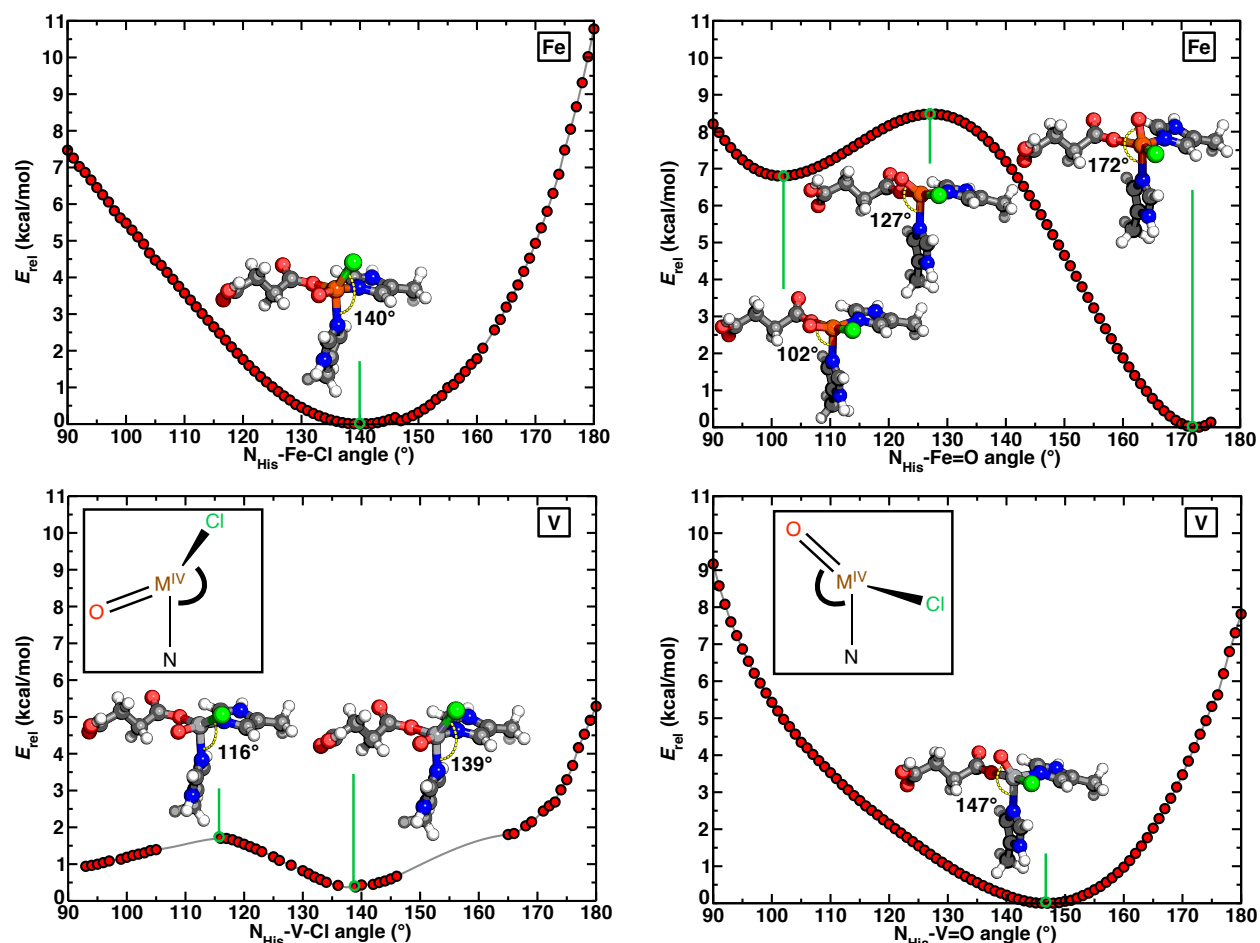


Figure 4. Reaction coordinates for isomerization between equatorial chloride and axial chloride (left), and equatorial oxo and axial oxo (right) with monodentate succinate for Fe (top) and V (bottom) active sites. The geometries corresponding to minima and transition states are shown as insets. The $N_{\text{His}}-M-\text{Cl}$ and $N_{\text{His}}-M=\text{O}$ angles (in $^{\circ}$, $M=\text{Fe}$, V) are indicated as yellow dashed curves in the insets along with skeleton structures that show the corresponding angles in the bottom plots. Hydrogen, carbon, nitrogen, oxygen, chlorine, vanadium, and iron are shown in

white, gray, blue, red, green, silver, and brown, respectively.

The second isomerization RC we explore connects the isomers of equatorial Cl⁻ and axial Cl⁻ with equatorial oxo (Figure 4). We again model this RC with monodentate succinate required for flexibility, and we describe the RC as a function of N_{His}-M-Cl angle, with the His that is trans to the axial moiety (Figure 4). Here, the Fe(IV)=O intermediate has a single minimum energy structure with the Cl⁻ tilted (N_{His}-M-Cl angle: 140°) out of the equatorial plane (Figure 5). The V(IV)=O RC obtained by single point calculations of the structures on Fe(IV)=O RC shares a similar minimum energy structure with Fe (Figure 4). However, we observe a small approximate barrier (ca. 2 kcal/mol) for V to the global minimum from equatorial Cl⁻ that was absent for Fe (Figure 4). We characterize the transition state (N_{His}-V-Cl angle: 116°) on the V(IV)=O RC by observing a single imaginary frequency of 69 cm⁻¹ along the angular RC mode. If we instead allow all but the RC coordinate to relax for V(IV)=O, we observe significant differences from both the Fe(IV)=O RC and its isostructural V(IV)=O RC (Supporting Information Figure S9). Here, we observe a steep energy landscape with a global minimum energy structure (N_{His}-V-Cl angle: 172°) that is similar to the axial Cl⁻ isomer (Supporting Information Figure S9).

Further examination of the relaxed Fe and V RCs reveals that the significant differences can be attributed to the position of oxo in the equatorial plane (Supporting Information Figures S10-S11). We observe that the angle formed by succinate, Fe, and oxo (O_{suc}-Fe=O) in the equatorial plane changes more significantly along the RC for V (86-145°) than for Fe (86-110°), resulting in an O_{suc}-V=O angle that is ca. 40° larger than the O_{suc}-Fe=O angle observed in the minimum energy structures along the relaxed RCs (Supporting Information Figure S10). This

indicates that potential isomerization from equatorial to axial Cl⁻ for halogenation would either be barrierless leading to axial Cl⁻ as global minimum or have a small energy barrier for the V(IV)=O mimics depending on the rigidity of the protein environment (Figure 4 and Supporting Information Figure S9). In contrast, for Fe, this transition is always barrierless and the minimum energy structure positions Cl⁻ midway between the equatorial plane and axial position (Figure 4). Thus, for systems where oxo is expected to be in the equatorial position during halogenation^{32, 113}, the behavior of non-heme Fe halogenases is not accurately captured by vanadium mimics which either predict a different minimum energy structure or the presence of a small energy barrier.

We also study an isomerization RC that corresponds to isomerization between equatorial hydroxo and axial hydroxo in which Cl⁻ remains equatorial. This isomerization is captured by the change in N_{His}-M-OH angle with the His trans to the axial hydroxo (Supporting Information Figure S12). While both Fe and V RCs are qualitatively similar with one global minimum, the minimum energy structures exhibit different N_{His}-M-OH angles, with the Fe intermediate having a ca. 10° larger angle than V intermediate (Supporting Information Figure S12). Comparison of this RC with the one connecting equatorial and axial oxo reveals significant differences for Fe but qualitatively similar landscapes for V (Figure 4 and Supporting Information Figure S12). This indicates that while axial oxo is preferred during isomerization prior to hydrogen atom transfer, the isomerization prior to halogenation prefers a geometry with the hydroxo tilted away (N_{His}-Fe-OH = 154°) from the axial orientation.

Overall, we observe that angular RCs and energy landscapes differ significantly between Fe and V systems, likely due to the differences in spin states for HS quintet Fe(IV)=O and LS doublet V(IV)=O intermediates, suggesting that observations of an isomer in a crystal structure

with the vanadyl mimic may not be reflective of the favored intermediate structure during catalysis. Our observations also suggest that hydrogen atom transfer to form the metal-hydroxo alters isomer stability in a fashion for Fe that also cannot be captured by V mimics of this intermediate. This highlights the limits of using LS/IS vanadium-based intermediates as mimics in experimental studies of fleeting HS iron intermediates in non-heme iron enzymes.

3.3 Binding Strength of Co-Substrates in Fe and V Active Sites.

Formation of a monodentate succinate is critical to isomerization for M(IV)=O and M(III)-OH intermediates (see Sec. 3.2), but qualitatively bidentate succinate configurations are generally more stable than monodentate succinate for both Fe and V intermediates (Figure 3). To quantify these differences in both binding configurations and with metal centers, we evaluated a quantum mechanical metric of bond order (i.e., the Mayer bond order¹¹⁴) to determine relative binding strengths of different orientations of succinate in comparison to a consistently bidentate α KG.

Although α KG is always bound in a bidentate fashion, its binding strength can vary by intermediate. The binding strength of α KG to Fe in HS O₂-isomers is twice that observed for HS water isomers (1.23 vs 0.49), suggesting a key role of the axial moiety in determining the binding strength of α KG (Figure 5). We observe a similar trend for ground spin state V intermediates, where α KG binds more strongly to V in LS O₂-isomers than in IS H₂O-isomers (1.69 vs 0.92, Figure 5 and Supporting Information Figure S13). Overall, α KG binds more strongly to V than Fe in corresponding intermediates, likely due to the preferred LS/IS ground state for V which leads to stronger binding than that observed in Fe intermediates with HS ground state (Figure 5).

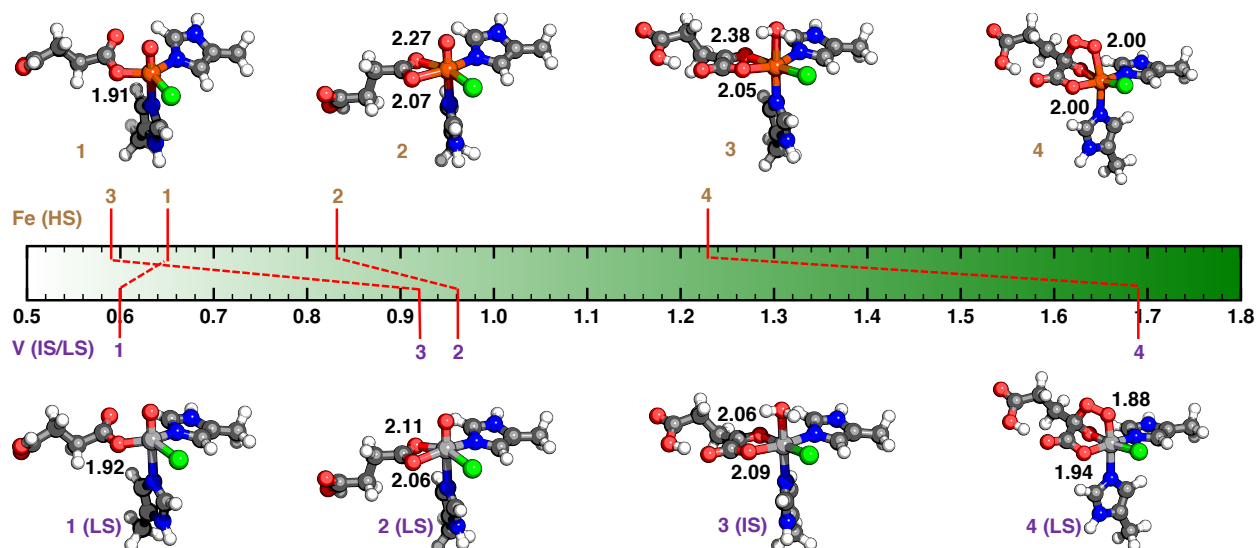


Figure 5. Bond order scale demonstrating the bond orders of metal-succinate bonds of ground state high-spin (HS) Fe (top) and intermediate-spin/low-spin (IS/LS) V (bottom) intermediates with monodentate and bidentate succinate. The four intermediates shown in the figure are (1) M(IV)=O with monodentate succinate, (2) M(IV)=O with bidentate succinate, (3) M(II)-H₂O with bidentate α KG, and (4) M(III)-O₂ with bidentate α KG. The corresponding M-O (of succinate/ α KG) bond lengths (in Å) are indicated in the insets. Hydrogen, carbon, nitrogen, oxygen, chlorine, vanadium, and iron are shown in white, grey, blue, red, green, silver, and brown, respectively.

Since bidentate-to-monodentate variations are expected to be most critical in isomerization of the M(IV)=O intermediate, we quantified the relative bond order in these intermediates for M = V and Fe. The bond order of not just monodentate (0.65) but also bidentate succinate (0.83) is significantly lower than that of α KG (1.23) for the HS Fe case (Figure 5). The difference in bidentate vs monodentate bond order (0.96 vs 0.60) is larger for LS V(IV)=O but bidentate binding strength is still markedly reduced in comparison to α KG (1.69, Figure 5). This difference could be why formation of the monodentate succinate V(IV)=O intermediate is less favorable than for the Fe(IV)=O case. These differences in bond order could also be anticipated in part based on differences in structure. The M–O distances of Fe/V to both O atoms of α KG are shorter by ca. 0.17 Å in comparison to average M–O distances for bidentate

succinate (Supporting Information Table S8). One of the Fe–O distances for bidentate succinate is much longer than the other (0.20 Å), suggesting an asymmetry in the bond strength, while the difference between V–O distances of bidentate succinate is smaller (0.05 Å, Supporting Information Table S8). While our analysis focuses on the M(IV)=O intermediate evaluated with gas-phase optimized geometries, similar trends hold for the M(III)–OH intermediate formed after HAT as well as upon inclusion of solvent during optimization of intermediates (Supporting Information Figures S14–S17). We thus conclude that the qualitatively bidentate succinate is only quantitatively slightly stronger bound than monodentate succinate in the case of Fe intermediates. This supports the potential isomerization between isomers of M(IV)=O and M(III)–OH intermediates to enable HAT and/or halogenation during the catalytic cycle (see Sec. 3.2).

We next sought to further isolate the effect of bidentate coordination on restricting isomerization of the reacting moiety. We created an artificial isomerization RC with the succinate constrained to its bidentate orientation and compare it to the RC we computed earlier with monodentate succinate (Figures 4 and 6). While the difference between monodentate and bidentate succinate isomers in free optimizations is ca. 3 kcal/mol for Fe and 18 kcal/mol for V, the endpoints (i.e., minima) of the RCs with bidentate succinate enforced are even further stabilized, especially for Fe (ca. 20 kcal/mol for Fe and 26 kcal/mol for V, Figures 3 and 6). Closer examination reveals that our enforcing the monodentate succinate constraint causes the C–O–M angle formed by succinate with the metal to be much larger than the angle observed in free optimizations (by ca. 47° for Fe and 38° for V) (Supporting Information Figure S18). We indeed observe that the average bond order of geometries along the RC obtained with monodentate succinate is smaller than that of free optimizations (Fe: 0.45 vs 0.65, V: 0.42 vs

0.60) likely due to the unfavorable C–O–M angle as a consequence of the constraints employed to separately probe a fully monodentate structure (Supporting Information Figure S19). Thus, partial bidentate-like character could be occurring during the unconstrained RC that has been more fully eliminated in our constrained approach.

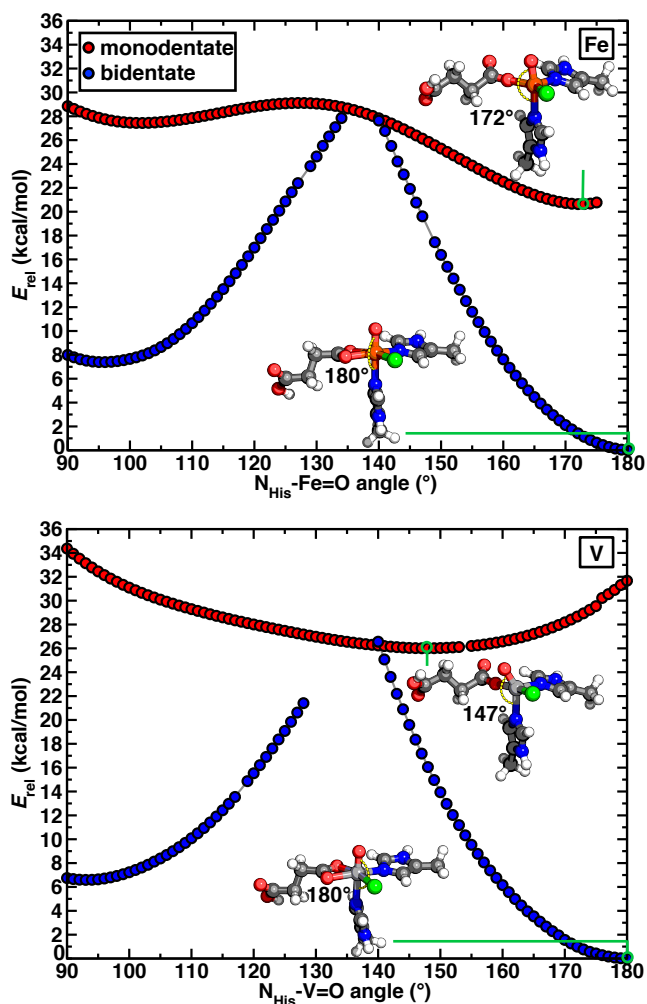


Figure 6. Reaction coordinates for isomerization between equatorial oxo and axial oxo with monodentate succinate (shown in red) and bidentate succinate (shown in blue) for Fe (top) and V (bottom) active sites. The geometries corresponding to minima are shown as insets. The N_{His} -M=O angle (in $^{\circ}$, M=Fe, V) is indicated as yellow dashed curves in the insets. Hydrogen, carbon, nitrogen, oxygen, chlorine, vanadium, and iron are shown in white, gray, blue, red, green, silver, and brown, respectively.

While the bidentate succinate is more stable than its monodentate counterpart in local minima, qualitatively bidentate structures are not likely to be feasible throughout the isomerization pathway with either Fe or V (Figure 6). Once the pathway reaches an $N_{\text{His}}\text{-M=O}$ angle of around $130\text{-}140^\circ$, bidentate structure can no longer be preserved (Figure 6 and Supporting Information Figure S20). Thus, isomerization is likely facilitated by the partial weakening of a qualitatively bidentate succinate only temporarily in the RC that causes a lower energetic penalty than fully breaking the second, weaker metal-succinate bond. We indeed observe that the bond orders of geometries along the RC obtained with bidentate succinate decrease slightly in moving from the global minima (ca. 0.85) to an $N_{\text{His}}\text{-M=O}$ angle of 140° (ca. 0.80), indicating that one of the M-O_{suc} bonds becomes weaker (Supporting Information Figure S19). However, we see an increase in bond order in moving from an $N_{\text{His}}\text{-M=O}$ angle of 100° (ca. 0.78) to 130° (ca. 0.88) as a result of constraining O_{suc} atoms to ensure bidentate succinate for these geometries (Supporting Information Figure S19). In the absence of these constraints, the M-O_{suc} bond order would likely decrease due to weakening of one of the M-O_{suc} bonds.

4. Conclusions.

While vanadyl is frequently used as a mimic for fleeting Fe(IV)=O in C-H activation, we showed through a combination of DFT geometry optimizations and accurate WFT energetics that there are crucial differences between ferryl and vanadyl intermediates that could be rationalized in terms of differences in their ground spin states. Crucially, the most favorable bidentate M(IV)=O isomer is different for Fe and V, and we also found that conversion between monodentate and bidentate isomers of the key metal-oxo/hydroxo intermediates is energetically favorable for Fe but strongly unfavorable in vanadyl mimics.

To further distinguish between V and Fe intermediates, we studied active site isomerization of monodentate Fe(IV)=O between equatorial and axial oxo isomers, and observed local and global minima for these isomers, separated by a transition state (ca. 8 kcal/mol). The corresponding RC for V(IV)=O is significantly different and lacks this barrier, with only one minimum where oxo is located midway between the equatorial plane and axial position. Additionally, we observed that Fe RC between equatorial and axial Cl⁻ isomers has a minimum energy structure that positions Cl⁻ midway between equatorial plane and axial position. However, isomerization on the corresponding V structure can be either barrierless with axial Cl⁻ as global minimum or have a small energy barrier (ca. 2 kcal/mol) depending on the protein environment rigidity. Contrary to M(IV)=O RCs that are significantly different for Fe and V, Fe(III)-OH and V(III)-OH have qualitatively similar energy landscapes but differing minimum energy structures. The significant differences in Fe and V RCs highlight the limits of using LS/IS V-based intermediates as mimics in studies of fleeting HS Fe intermediates in non-heme iron enzymes.

Since isomerization requires the formation of monodentate succinate, we analyzed the relative bond strengths of monodentate and bidentate succinate for Fe and V. We quantified the succinate binding strength to the metal to understand the differences in monodentate and bidentate succinate binding relative to bidentate α KG. We observed that α KG binds more strongly to V than Fe, likely due to the preferred LS/IS ground state for V. Furthermore, for both Fe and V, bidentate succinate binds with greater strength than monodentate succinate, but weaker than α KG. The RC with bidentate succinate revealed that bidentate structures are not accessible throughout the RC, indicating a weaker metal-succinate bond must form during isomerization. This is indeed supported by the decreased binding strength of bidentate succinate along the RC away from the global minimum.

Overall, our study highlights the limits of using V-based intermediates as mimics in non-heme iron halogenases owing to differences in spin states that result in different geometries, isomer energetics, and isomerization RCs. Moreover, quantifying the binding strength of succinate sets the stage for further understanding the active site isomerization in non-heme iron halogenases.

5. Computational Details.

The active site of a representative non-heme iron halogenase, WelO5^{32, 111-112} (PDB ID: 5IQS), was extracted from the enzyme's crystal structure. The model comprised the Fe metal center, chloride, α KG, water, and two metal-bound His ligands truncated at the sidechain (i.e., excluding C α and backbone atoms, Supporting Information Figure S21). Hydrogen atoms were added to the extracted active site using Avogadro v1.2.0¹¹⁵, and the metal-distal carboxylate oxygen of α KG and N δ atoms of His ligands were also protonated (Supporting Information Figure S21). The final active site model had an overall neutral charge. All added hydrogen atoms were optimized with the UFF force-field¹¹⁶ while the heavy atoms were held fixed. The isomers of the active site containing water, O₂, oxo, hydroxo, and succinate (monodentate and bidentate with succinate tail protonated) ligands were generated with molSimplify¹¹⁷, which uses OpenBabel¹¹⁸⁻¹¹⁹ as a backend. The iron metal center was replaced by vanadium to generate similar initial geometries with a vanadium metal center.

All constrained geometry optimizations were performed with density functional theory (DFT) using the generalized gradient approximation (GGA) global hybrid PBE0¹²⁰ (with 25% exchange) and the def2-TZVP basis set¹²¹ using ORCA¹²²⁻¹²³ v.4.0.1.2 and v.4.2.1. For these optimizations, PBE0 was augmented with the semi-empirical D3¹²⁴ dispersion using Becke-

Johnson¹²⁵ damping. All optimizations were carried out in redundant internal coordinates using the BFGS algorithm with default thresholds of 3×10^{-4} hartree/bohr for the maximum gradient and 5×10^{-6} hartree for the change in self-consistent field (SCF) energy between steps. For all optimizations, the methyl carbon atoms of His ligands and the five heavy atoms of succinate were held fixed to mimic the ligand positions in the enzyme (Supporting Information Figure S22). Singlet calculations were carried out in a spin-restricted formalism while all other spin-states were simulated as unrestricted calculations (Supporting Information Tables S1–S2).

Geometry optimizations of the active site isomers were carried out in both gas-phase and solvent-phase with two solvent dielectric values, $\epsilon = 10$ and 80 , approximately mimicking the protein and an aqueous environment, respectively. The solvent-phase optimizations were carried out using the conductor-like polarizable continuum model¹²⁶ (C-PCM) solvation energies in combination with the conductor-like screening solvent model (COSMO) epsilon function type obtained at the PBE0/def2-TZVP level of theory. All initial and optimized structures for both gas- and solvent-phase optimizations are provided in the Supporting Information.

Initial geometries to generate RCs were constructed from the PBE0/def2-TZVP optimized geometries of active site isomers using an in-house Python script. The isomerization RCs were sampled by rotating oxo and/or chloride ligands with respect to the axial His ligand in 1° increments of the angle formed by oxo/chloride, metal center (i.e., Fe, V), and nitrogen of the axial His (Supporting Information Text S4 and Figure S23). This was followed by constrained optimizations along the chosen RC for both iron and vanadium metal centers (Supporting Information Text S4). Single point calculations were also carried out at PBE0/def2-TZVP level of theory on optimized geometries with iron metal center by replacing iron with vanadium to understand the effect of different metal centers for a given geometry. High-energy structures

along the PBE0/def2-TZVP optimized RCs were used to obtain vibrational frequencies. Numerical Hessian calculations were carried out at the PBE0/def2-TZVP level of theory where the Hessian was computed using the central difference approach after $6N$ atomic displacements with ORCA v.4.0.1.2. The presence of an imaginary frequency along the RC confirmed that the high-energy structure corresponded to a transition-state.

Single point energy calculations on the PBE0/def2-TZVP geometries of various active site isomers were carried out at the domain-localized pair natural orbital coupled cluster level of theory with single, double and perturbative triples (i.e., DLPNO-CCSD(T)¹²⁷⁻¹²⁸) using ORCA v4.0.1.2. Dunning-style correlation consistent double- ζ and triple- ζ (i.e., aug-cc-pVDZ and aug-cc-pVTZ) basis sets were employed to enable two-point¹²⁹⁻¹³¹ extrapolation to the complete basis set (CBS) limit. All reported DLPNO-CCSD(T) energies were obtained using tight PNO thresholds (Supporting Information Table S9). Multiwfn¹³² was used to perform Mayer bond order analysis to quantify the strength of binding of α KG, monodentate, and bidentate succinate to both iron and vanadium metal centers.

ASSOCIATED CONTENT

Supporting Information. Isomers of intermediates of the non-heme iron halogenase active site; potential spin states of Fe and V active site intermediates; geometries of different spin states for Fe and V active site intermediates; optimized geometries of Fe(IV)=O in different spin states; DLPNO-CCSD(T)/CBS spin splitting energies for isomers of all intermediates; optimized metal-ligand bond lengths of representative isomers of Fe and V; Mayer's bond orders of representative active site isomers of Fe and V; isomers of V(IV)=O showing elongated V–N_{His} or V–O_{suc} bonds; differences between solvent-phase and gas-phase metal-ligand bond lengths; gas-phase and solvent-phase optimized geometries of the Fe(IV)=O isomer; convergence of isomers to appropriate geometries; initial and optimized geometries of M(IV)=O and M(III)–OH isomers; differences between IS Fe and LS V metal-ligand bond lengths of M(IV)=O; DLPNO-CCSD(T)/CBS energies of IS Fe(IV)=O and LS V(IV)=O isomers; comparison of IS/LS Fe intermediates to IS/LS V intermediates; optimized geometries of M(II)–H₂O and M(III)–O₂

isomers; DLPNO-CCSD(T)/CBS relative energies of M(II)-H₂O, M(III)-O₂ isomers; isomerization RC between equatorial and axial oxo for V(IV)=O; isomerization RC between equatorial and axial Cl⁻ for V(IV)=O; O_{suc}-M=O angle as a function of isomerization RC angle N_{His}-M-Cl⁻; V(IV)=O isomerization RC between eq. and ax. Cl⁻ with extra constraints; isomerization RC between equatorial and axial hydroxo for M(III)-OH; bond order scale of HS Fe and LS V intermediates in gas-phase; metal-ligand bond lengths for intermediates shown in Figure 5; bond order scale of ground spin state Fe and V intermediates in gas-phase; bond order scale of HS Fe and LS V intermediates in gas-phase; bond order scale of the ground state Fe and V intermediates in solvent-phase; bond order scale of HS Fe and LS V intermediates in solvent-phase; stable M(IV)=O geometries from free and constrained optimizations; M-O bond orders of geometries along Fe and V isomerization RCs; representative structure along isomerization RC; extracted active site of WelO5; representative structure showing constrained atoms in optimizations; details of reaction coordinate construction; representative structures showing reaction coordinate angles; tight PNO default thresholds used for DLPNO-CCSD(T) calculations (PDF)

Initial geometries, gas-phase optimized geometries, and solvent-phase optimized geometries of isomers of Fe and V active site intermediates; initial and optimized geometries along Fe and V isomerization reaction coordinates for equatorial and axial oxo isomerization as well as equatorial and axial chloride isomerization (ZIP)

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Notes

The authors declare no competing financial interest.

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