A Synthetic Model of the Putative Fe(II)-Iminobenzosemiquinonate Intermediate in the Catalytic Cycle of o-Aminophenol Dioxygenases

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A Synthetic Model of the Putative Fe(II)-Iminobenzosemiquinonate Intermediate in the Catalytic Cycle of o-Aminophenol Dioxygenases

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Abstract

The oxidative ring cleavage of aromatic substrates by nonheme Fe dioxygenases is thought to involve formation of a ferrous–(substrate radical) intermediate. Here we describe the synthesis of the trigonal-bipyramidal complex Fe(Ph₂Tp)(ISQᵗBu) (2), the first synthetic example of an iron(II) center bound to an iminobenzosemiquinonate (ISQ) radical. The unique electronic structure of this $S = 3/2$ complex and its one-electron oxidized derivative ([3]+) have been established on the basis of crystallographic, spectroscopic, and computational analyses. These findings further demonstrate the viability of Fe²⁺–ISQ intermediates in the catalytic cycles of o-aminophenol dioxygenases.

In biochemical pathways, the oxidative ring cleavage of substituted aromatic compounds, such as catechols and o-aminophenols, is generally performed by mononuclear nonheme iron dioxygenases.¹ While these enzymes are usually found in bacteria, some play important roles in human metabolism: for instance, a key step in tryptophan degradation involves the O₂-mediated ring cleavage of 3-hydroxyanthranilate (HAA) by HAA-3,4-dioxygenase (HAD; Scheme 1).² With the exception of the intradiol catechol dioxygenases, the ring-cleaving dioxygenases share a common O₂-activation mechanism, illustrated in Scheme 2.¹ A notable feature of this proposed mechanism is the superoxo-Fe²⁺-(iminobenzo)semiquinonate intermediate (B) that is thought to form after O₂ binding to the enzyme–substrate complex (A). The development of radical character on the substrate ligand presumably facilitates reaction with the bound superoxide, yielding the key Fe²⁺-alkylperoxo intermediate (C).³ Although the electronic structure of B remains somewhat controversial,⁴ evidence in favor of substrate radical character has been provided by radical-trap experiments⁵ and DFT calculations,³ as well as a remarkable X-ray structure of the Fe/O₂ adduct of an
extradiol dioxygenase in which the radical character of the bound substrate was inferred from its nonplanar geometry.\(^6\)

\[
\text{3-hydroxyxanthranilate (HAA)}
\]

**Scheme 1.** Reaction Catalyzed by HAA Dioxygenase (HAD)

Despite these biological precedents, synthetic analogues of intermediate B in which a ferrous center is coordinated to an (iminobenzo)semiquinone radical, (I)SQ, have been lacking in the literature, even though numerous ferric complexes with such ligands exist.\(^7^-^1^1\) Herein, we report the synthesis and detailed characterization of an Fe\(^{2+}\)–ISQ complex, 2, that represents the first synthetic model of this important type of enzyme intermediate. We also examine the geometric and electronic structures of the species [3]\(^+\) generated via one-electron oxidation of 2.

\[
\text{Scheme 2. Catalytic Cycle of Ring-Cleaving Dioxygenases}
\]

In our efforts to generate synthetic models of HAD, we have used the tris(pyrazolyl)borate ligand, \(^{\text{Ph}_2}\text{Tp},\(^{12}\) to mimic the facial His\(_2\)Glu coordination environment of the enzyme active site. The reaction of \([\text{(Ph}_2\text{Tp})\text{Fe(OBz)}]\)^\(^{13}\) with 2-amino-4,6-di-tert-butyphenol (\(^{\text{tBu}}\text{APH})\) in the presence of base provided the light yellow complex \([\text{(Ph}_2\text{Tp})\text{Fe}^{2+} (^{\text{tBu}}\text{APH})]\) (1) in 71% yield. The X-ray crystal structure of 1 reveals a five-coordinate (5C) Fe\(^{2+}\) center in which the \(^{\text{tBu}}\text{APH}^-\) ligand binds in a bidentate fashion (Figure 1; crystallographic details are

\(\text{H}^+\)
summarized in Table S1 in the Supporting Information). The average Fe1–N_{Tp} bond length of 2.15 Å is typical of high-spin Fe^{2+} complexes with Tp ligands,^{13,14} while the short Fe1–O1 distance of 1.931(1) Å is consistent with coordination by an aminophenolate anion (Table 1). The complex adopts a distorted trigonal-bipyramidal geometry (τ = 0.61^{15}) with the amino group of t^{18}bAPH in an axial position trans to N5. To the best of our knowledge, 1 represents the first synthetic model of an aminophenol dioxygenase.

**Figure 1.** Synthesis and thermal ellipsoid diagram of complex 1. For the sake of simplicity, the 5-Ph substituents of the Ph_{2}Tp ligand have been omitted and only the amino hydrogens are shown. Selected bond lengths are provided in Table 1.

Reaction of 1 with 1 equiv of 2,4,6-tri-tert-butylphenoxy radical (TTBP*) at RT in CH_{2}Cl_{2} gives rise to a distinct chromophore, 2, with a broad absorption manifold centered at 715 nm (ε_{max} = 0.76 mM^{-1} cm^{-1}; see Figure 2). Addition of MeCN, followed by cooling to −30 °C, provides pale green crystals of 2 suitable for crystallographic analysis. As with 1, the X-ray structure of 2 features a neutral 5C Fe complex with a distorted trigonal-bipyramidal geometry (τ = 0.58), although O1 now occupies an axial position instead of N7 (Figure S1). The N7 atom in 2 is monoprotonated, confirming that 2 is generated via abstraction of a H-atom from the −NH_{2} group of 1.
Figure 2. Electronic absorption spectra of 1 (---), 2 (--), and [3]SbF$_6$ (- - -) measured in CH$_2$Cl$_2$ at RT.

Interestingly, the average Fe1–N$_{Tp}$ bond distance observed for 2 (2.136 Å) is nearly identical to the value found for 1 (2.150 Å), suggesting minimal change in Fe charge. Metric parameters for the O,N-coordinated ligand, however, are dramatically different in the two structures. In the structure of 1, the six C–C bonds of the tBuAPH$^-$ ring are approximately equidistant (1.40 ± 0.02 Å), reflecting its closed-shell, aromatic nature. In contrast, the corresponding C–C bond distances in 2 exhibit the “four long/two short” distortion commonly observed for quinoid moieties (Table 1).7-11 The short O1–C1 and N7–C2 distances of 1.285(3) and 1.328(4) Å, respectively, are also characteristic of ISQ$^-$ ligands, as amply demonstrated by Wieghardt8-10 and others.7 Thus, the X-ray crystallographic data strongly support the formulation of 2 as [(Ph$_2$Tp)Fe$_{2+}$(tBuISQ)]. This assignment rationalizes the absorption spectrum of 2, which closely resembles those reported for Co$_{3+}$ and Ni$_{2+}$ complexes with a lone ISQ$^-$ ligand.9a

The X-band EPR spectrum of 2 displays an intense peak at $g = 6.5$, along with a broad derivative-shaped feature centered near $g = 1.8$ (Figure 3). Such spectra are typical of $S = 3/2$ systems with large and rhombic zero-field splitting parameters.9,16 The simulated spectrum in Figure 3 assumed a negative $D$-value (with $|D| > h
u$), an $E/D$-ratio of 0.24, and $g$-values of 2.36, 2.30, and 2.17. Significant $E/D$ strain was incorporated to adequately account for the broadness.
of the higher-field features. The combined experimental results therefore indicate that 2 contains a high-spin Fe$^{2+}$ center ($S = 2$) antiferromagnetically coupled to a $^t$BuISQ radical anion.

**Table 1.** Selected Bond Distances (Å) for Complexes 1–3

<table>
<thead>
<tr>
<th></th>
<th>Complex 1</th>
<th>Complex 2</th>
<th>[3]SbF$_6$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe1–N1</td>
<td>2.101(1)</td>
<td>2.108(2)</td>
<td>2.071(7)</td>
</tr>
<tr>
<td>Fe1–N3</td>
<td>2.127(1)</td>
<td>2.087(2)</td>
<td>2.038(7)</td>
</tr>
<tr>
<td>Fe1–N5</td>
<td>2.223(1)</td>
<td>2.216(2)</td>
<td>2.134(6)</td>
</tr>
<tr>
<td>Fe1–O1</td>
<td>1.931(1)</td>
<td>2.095(2)</td>
<td>2.082(6)</td>
</tr>
<tr>
<td>Fe1–N7</td>
<td>2.214(1)</td>
<td>1.982(2)</td>
<td>2.017(8)</td>
</tr>
<tr>
<td>O1–C1</td>
<td>1.345(2)</td>
<td>1.285(3)</td>
<td>1.26(1)</td>
</tr>
<tr>
<td>N7–C2</td>
<td>1.451(2)</td>
<td>1.328(4)</td>
<td>1.33(1)</td>
</tr>
<tr>
<td>C1–C2</td>
<td>1.398(2)</td>
<td>1.469(5)</td>
<td>1.47(1)</td>
</tr>
<tr>
<td>C2–C3</td>
<td>1.388(2)</td>
<td>1.413(4)</td>
<td>1.42(1)</td>
</tr>
<tr>
<td>C3–C4</td>
<td>1.388(2)</td>
<td>1.363(4)</td>
<td>1.35(2)</td>
</tr>
<tr>
<td>C4–C5</td>
<td>1.403(2)</td>
<td>1.427(4)</td>
<td>1.43(2)</td>
</tr>
<tr>
<td>C5–C6</td>
<td>1.394(2)</td>
<td>1.375(4)</td>
<td>1.37(2)</td>
</tr>
<tr>
<td>C1–C6</td>
<td>1.420(2)</td>
<td>1.440(4)</td>
<td>1.44(1)</td>
</tr>
</tbody>
</table>

$^a$The bond distances listed here represent the average distance in the two independent units of [3]$^+$, while the uncertainty is taken to be the larger of the two σ-values.

Further evidence in favor of a ligand-based radical was obtained from density functional theory (DFT) calculations. Two geometry-optimized models of 2 with $S = 3/2$ were computed that differ with respect to their electronic configurations. Analysis of the geometric and electronic structure of the first model (2$_A$) indicates that it contains an intermediate-spin Fe$^{3+}$ center coordinated to a closed-shell imidophenolate ligand, $^t$BuAP$^{2-}$. The optimized structure of 2$_A$ features a square-pyramidal geometry ($τ = 0.18$) with very short Fe–O1 and Fe–
N7 distances of \( \sim 1.87 \text{ Å} \), in poor agreement with the experimental structure (Table S2). Furthermore, the computed bond distances for the \( \text{tBuAP}^2^- \) ligand deviate sharply from the distances found experimentally for \( \text{2} \), with nearly all such differences being significantly greater than the estimated error (3\( \sigma \)) in the crystallographic data. The second model (\( \text{2B} \)) was generated via a broken-symmetry calculation in order to obtain the \([((\text{Ph}^2\text{Tp})\text{Fe}^{2+}(\text{tBuISQ})] \) electronic configuration described above. The resulting structure accurately reproduces the overall trigonal-bipyramidal geometry of \( \text{2} \) and provides reasonably consistent Fe–ligand distances. Most importantly, the computed and experimental \( \text{tBuISQ}^- \) bond distances exhibit remarkable agreement, with an rms deviation of merely 0.007 Å (Table S2). Model \( \text{2B} \) is also 9 kcal/mol more stable than \( \text{2A} \), indicating an energetic preference for the \( \text{Fe}^{2+}–\text{tBuISQ} \) form.

Figure 3. X-band EPR spectrum of \( \text{2} \) at 20 K. The derivative-shaped feature at \( g = 4.3 \) (▼) arises from a minor ferric impurity, while the feature at \( g = 2.0 \) (*) is due to a residual TTBP radical. Parameters used to generate the simulated spectrum are provided in the text.

To the best of our knowledge, the electronic structure of \( \text{2} \) has no precedent among synthetic complexes. While \( \text{Fe}^{2+}–\text{SQ} \) intermediates are often invoked in the mechanisms of catechol dioxygenases, all relevant models to date feature unambiguous \([\text{Fe}^{3+}–\text{catecholate}]^+ \) units.\(^{17,18} \) Similarly, the \( \text{Fe}^{3+}–\text{ISQ} \) complexes generated by Wieghardt and co-workers exclusively undergo ligand-based reductions to give the corresponding \( \text{Fe}^{3+}–\text{AP} \) species.\(^{8,9} \) The unique
Fe$^{2+}$–ISQ configuration of 2 is likely due to the presence of a high-spin, 5C Fe ion, whereas related complexes prepared by Wieghardt (such as [[(L)Fe$^{3+}$][RISQ]]$^+$, where L = cis-cyclam and R = H or tBu) generally feature low-spin, 6C Fe centers. Thus, changes in spin state and coordination geometry are capable of shifting the delicate balance between the Fe$^{2+}$–ISQ and Fe$^{3+}$–AP valence tautomers.

Reaction of 2 with 1 equiv of an acetylferrocenium salt in CH$_2$Cl$_2$ provides a dark green species, [3]$^+$, with intense absorption features at 770 and 430 nm (Figure 2). Treatment of [3]$^+$ with 1 equiv of reductant (such as Fe(Cp*)$_2$) fully regenerates 2 (Figure S2), indicating that the two species are related by a reversible one-electron process. EPR experiments with frozen solutions of [3]$^+$ failed to detect a signal in either perpendicular or parallel mode, indicative of an integer-spin system. Indeed, the magnetic moment of [3]$^+$ was found to be 5.0(1) $\mu_B$ at RT, close to the spin-only value for an $S = 2$ paramagnet.

X-ray quality crystals of [3]SbF$_6$ were prepared by vapor diffusion of pentane into a concentrated dichloroethane solution. The resulting structure (Figure S3) contains two symmetrically independent Fe units, each featuring a distorted square-pyramidal geometry (τ = 0.42 and 0.38). Despite the difference in charge, complexes [3]$^+$ and 2 have identical atomic compositions. Yet the average Fe–N$_{Tp}$ bond distance shortens from 2.132 to 2.081 Å upon conversion of 2 to [3]$^+$, suggesting an increase in Fe-based charge. While the structural parameters of the bidentate O,N-donor ligand of [3]$^+$ are consistent with a tBuISQ$^-$ radical, it was not possible to rule out a neutral iminobenzoquinonate ligand (tBuIBQ) due to sizable uncertainties in the bond distances.

We therefore turned to DFT calculations to further explore the electronic structure of [3]$^+$. The resulting geometry-optimized model, [3$_{DFT}$$^+$, exhibits good agreement with the crystallographic data, although the DFT structure is more distorted toward the trigonal-bipyramidal limit (τ = 0.64; Table S3). The computed Fe–ligand bond distances nicely match the experimental values (rms deviation = 0.022 Å), indicating that the calculation converges to the correct $S = 2$ electronic configuration. Comparison of [3$_{DFT}$$^+$ and 2$_B$ reveals more pronounced “quinoid” character in the O,N-donor ligand of the former.
Using the experimentally derived correlations of bond distances and ligand oxidation states recently published by Brown, the $O,N$-donor ligand of $[3_{\text{DFT}}]^+$ has an oxidation state of $-0.35(5)$ (i.e., partway between ISQ$^1$- and IBQ$^0$). Moreover, the highest-occupied spin-down MO ($\beta$-HOMO) of $[3]^+$ contains roughly equal Fe and ligand character (47 and 42%, respectively), and the $\beta$-LUMO is evenly delocalized over the two units (Figure S4). Thus, the DFT results suggest that the electronic structure of $[3]^+$ lies between the Fe$^{3+}$–$^{tBu}$ISQ and Fe$^{2+}$–$^{tBu}$IBQ limits. Detailed spectroscopic studies are currently underway to better understand the electronic structure of $[3]^+$.

Complexes 1–3 replicate key structural and electronic aspects of the proposed o-aminophenol dioxygenase mechanism. In particular, the conversion of 1→2 mimics the transformation of the enzyme–substrate complex (A) into a ferrous–ISQ species (B) via coupled proton and electron transfers. Our results therefore provide a synthetic precedent for the existence of Fe$^{2+}$–ISQ intermediates in enzymatic catalysis. Of course, complex 2 is an imperfect model of intermediate B, since it lacks the coordinated superoxo ligand. Attempts are currently in progress to characterize species formed during the reaction of 1 and 2 with O$_2$ (and its surrogate, NO). These studies will yield further insights into the role of noninnocent ligands in ring-cleaving dioxygenase mechanisms.

**Supporting Information**

Experimental details, computational methods and models, crystallographic structures and data (CIFs), and absorption spectra of the interconversion of 2 and $[3]^+$. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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12 Abbreviations: Ph²Tp = hydrotris(3,5-diphenylpyrazol-1-yl)borate(1−). °AP = o-imidophenolate(2−) anion with R-groups at the 4- and 6-positions. °ISQ = o-iminobenzosemiquinone(1−) with R-groups at the 4- and 6-positions.


The low-energy catecholate→Fe$^{3+}$ charge transfer transitions (700–900 nm) exhibited by some models point to a low-lying excited state with Fe$^{2+}$–SQ character. Interaction with this excited state may introduce a small amount of SQ character into the ground state.


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