**Nucleophilic Reactivity of a Copper(II)-Superoxide Complex**

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**Abstract:** Metal-bound superoxide intermediates are often implicated as electrophilic oxidants in dioxygen activating metalloenzymes. In the nonheme iron α-ketoglutarate dependent oxygenases and pterin dependent hydroxylases, however, Fe\(^{II}\)-superoxide intermediates are postulated to react by nucleophilic attack on electrocyclic carbon atoms. By reacting with a Cu\(^{II}\)-superoxide complex (I) with acyl chloride substrates, we have found that a metal-superoxide complex can be a very reactive nucleophile. Furthermore, I was found to be an efficient nucleophilic deforming reagent, capable of Baeyer-Villiger oxidation of a number of aldehyde substrates. The observed nucleophilic chemistry represents a new domain for metal-superoxide reactivity. Our observations provide support for the postulated role of metal-superoxide intermediates in nonheme iron α-ketoglutarate dependent and pterin-dependent enzymes.

Metal-superoxide species have been implicated as reactive intermediates in the catalytic cycles of a variety of Cu- and Fe-containing metalloenzymes.\(^1\) In the Cu-hydroxylase enzymes, dopamine-β-hydroxylase (D/BH) and peptidylglycine α-hydroxylating monooxygenase (PHM), a Cu\(^{II}\)-superoxide moiety is postulated to act as an electrophilic hydrogen-atom abstraction (HAA) reagent.\(^2\) X-ray diffraction characterization of a Cu\(^{II}\)-superoxide species in PHM provided structural support for this hypothesis.\(^3\) In the nonheme iron enzyme superfamily, Fe\(^{II}\)-superoxide intermediates have been ascribed roles as both electrophilic and nucleophilic reactants. For example, in isopenicillin N synthase (IPNS), 2-hydroxyethylphosphonate dioxygenase (HEPD), 2-hydroxypropylphosphonic acid epoxide (HppE), 1-aminoacyclopropane-1-carboxylic acid oxidase (ACCO), an Fe\(^{II}\)-superoxide unit is proposed to perform HAA during O₂ activation.\(^4\) In contrast, in the α-ketoglutarate (α-KG) dependent oxygenases, an Fe\(^{II}\)-superoxide intermediate is postulated to react with the α-keto-carbon of α-KG by nucleophilic attack.\(^5\) Likewise, in the pterin-dependent hydroxylases a nucleophilic Fe\(^{II}\)-superoxide is proposed to attack an electrophilic carbon atom of pterin.\(^6\) An Fe\(^{II}\)-superoxide intermediate in a Rieske dioxygenase mutant was recently trapped and spectroscopically characterized\(^7\) however, rather than display electrophilic HAA or nucleophilic reactivity, this species decayed by electron transfer. Overall, strong experimental support for the role of enzymatic metal-superoxide intermediates acting as electrophilic oxidants exists, whereas little to no support for their postulated roles as nucleophiles has been reported to date.

A number of mononuclear Cu\(^{II}\)-superoxide model complexes have been trapped and characterized, allowing for an assessment of the reactivity of the metal-bound superoxide ligand.\(^11\) These complexes displayed the ability to effect a variety of transformations (HAA, electron transfer, O-atom transfer) that mimic the electrophilic chemistry of copper hydroxylases. Mononuclear Fe\(^{II}\)-superoxide complexes remain elusive, while a single example of a dinuclear Fe(III)-OH₂-Fe(II)-superoxide complex exists.\(^9\) This complex was capable of acting as an electrophile in the oxidation of phenols. Evidence for the involvement of Fe\(^{III}\)-superoxide intermediates in the activation of dioxygen by Fe\(^{II}\) model complexes exists, in one case the postulated Fe\(^{III}\)-superoxide species acted as an electrophilic HAA reagent.\(^10\) in the other as a presumed nucleophile.\(^11\) In general, metal-superoxide model complexes display electrophilic reactivity. To the best of our knowledge, no direct evidence for a metal-bound superoxide ligand reacting as a nucleophile has been described to date. As such, no experimental verification of the postulated nucleophilic reactivity of Fe\(^{III}\)-superoxide species in the α-KG and pterin-dependent nonheme iron enzymes exists. Herein we describe our investigations into the nucleophilic reactivity of a metal-superoxide model complex towards electrophilic carbonyls.

Of the metal-superoxide complexes that we tested, only Tolman’s [N,N'-Bis(2,6-diisopropylphenyl)-2,6-pyridinedicarboxamido]-superoxo-copper(II) complex\(^18\) (I, Scheme 1) was suitably reactive. Indeed, Tolman surmised I was likely a good nucleophile, given that it was readily protonated, and displayed poor HAA reactivity. The nucleophilic character of I was first tested in its reaction towards acyl chloride substrates,
electrophiles that commonly react with metal-peroxide complexes. I was reacted with benzoyl chloride (PhCOCI, 10 equiv.) at -80 °C in THF/DMF (3:1). An immediate reaction occurred resulting in the disappearance of the characteristic electronic absorption features (λ_{max} = 627 nm) assigned to I within 40 s (Figure 2). 1H NMR and GCMS analysis of the post-reaction products indicated the formation of benzoic acid. A pseudo-first-order rate constant (k_{obs}) for the reaction was calculated by plotting the change in absorbance intensity of the λ_{max} = 627 nm feature of I against time and fitting the resulting curve (Figure S1, see supporting information file). The second-order rate constant (k_{0}) was determined by plotting k_{obs}-values determined under a series of substrate concentrations followed by calculating the slope of the resulting linear plot (Figure 1, inset). The k_{0}-value determined for the reaction between I and PhCOCI (4.49 M^{-1}s^{-1}) was relatively high compared to k_{0}-values determined for the reaction between aldehyde electrophiles and nucleophlic peroxide-complexes (Table 1). This observation is not unexpected as the acyl chloride is anticipated to be more electrophilic than an aldehyde. I is thus a capable nucleophile that reacts rapidly with an electrophilic acyl chloride substrate at low temperatures.

In the reaction between I and acyl chlorides we determined k_{0}-values for a series of para-substituted benzoyl chloride substrates (p-R-PhCOCI, R = Bu, H, F, Cl, NO2). We hypothesized that a Hammett plot of the log(k_{0}/k_{ref}) versus the para-substituent Hammett parameter (σ_p) would be linear and yield a positive r-value, providing proof of a nucleophilic attack by I on p-R-PhCOCI. Indeed, Nam has utilized this approach to demonstrate the strong nucleophilic character that certain metal-peroxide complexes possess. However, the Hammett plot yielded a r-value close to 0 (Figure S7, all substrates displayed comparable k_{0}-values). These observations provide a strong indication that the rate-determining step in the reaction between I and acyl chlorides is not the nucleophilic attack of the superoxide ligand on the electrophilic carbonyl.

For a better understanding of the nucleophilic reactivity of I, we investigated its reactivity in aldehyde deformylation reactions. Previous reports have demonstrated metal-bound peroxide ligands are proficient deforming reagents. These studies have provided an understanding of the nucleophilic properties of a variety of metal-peroxide complexes. I reacted with 100 equivalents of 2-phenylpropanaldehyde (PPA) at -80 °C in THF/DMF (3:1) (Figure S8). The reaction was complete within 400 s, and GC-MS and 1H NMR analysis of the reaction mixture showed the formation of acetophenone. I also reacted with 100 equivalents of cyclohexancarboxaldehyde (CCA) at -80 °C in THF/DMF (3:1) (Figure S11). The reaction was complete within 20 s, and GC-MS and 1H NMR analysis of the reaction mixture showed the formation of cyclohexanone. The k_{0}-values determined for the reactions between I and PPA and CCA at -80 °C were 0.062 M^{-1}s^{-1} and 1.43 M^{-1}s^{-1} respectively (Figures S9 and S12). Further insight into the reaction between I and PPA/CCA was obtained by determining the activation enthalpy and entropy of the reactions. The ΔH^‡ values (PPA = 40 kJmol^{-1}; CCA = 35 kJmol^{-1}) were comparable to those determined for the reaction between PPA/CCA and Fe^{II} and Co^{II} peroxide complexes (Tables 1 and S1). The entropy of activation values (ΔS^‡; PPA = -63 JK^{-1}mol^{-1}; CCA = -80 JK^{-1}mol^{-1}) were large and negative, indicative of a bimolecular reaction (See figures S10 and S13). The obtained kinetic and thermodynamic parameters clearly demonstrate that I is an effective nucleophilic deformylating reagent that displays reactivity properties similar to those reported for nucleophilic deformylation reactions performed by metal-peroxide complexes.

Interestingly, comparison of the k_{0}-values determined for the reaction between metal-peroxide complexes and PPA/CCA with those established for I suggests that I is a very reactive oxidant (Tables 1 and S1). For PPA, at -80 °C I displayed a k_{0}-value (0.062 M^{-1}s^{-1}) comparable to those determined for Fe^{II}, Ni^{II}, and Co^{II} peroxide complexes at significantly higher temperatures (between -40 and 25 °C, k_{0} = 0.015 - 0.13 M^{-1}s^{-1}, Table 1). The only model complex that displayed higher deformation rates than I is [Fe^{III}(η<sub>2</sub>-ξ<sub>2</sub>-O)(TMCS)], which contains a peroxide dianion ligand that reacted with PPA at -90 °C at such high rates that accurate kinetic analysis was not possible. I is thus highly reactive compared to its metal-peroxide counterparts.

Previous studies have demonstrated that para-substituted benzaldehydes (p-R-PhCHO) can provide mechanistic insights into nucleophilic deformylation reactivity. For Mn^{II}, Fe^{II}, and Co^{II} peroxide complexes, a plot of log(k_{0}/k_{ref}) versus σ_p is linear, with a positive r-value. This observation indicated that the peroxide ligand in these complexes was reacting as a nucleophile. With this in mind, we investigated the reactivity of I towards p-R-PhCHO (R = H, NMe2, OMe, Me, Cl, NO2). However, no reaction was observed upon exposure of I to up to 100 equivalents of p-R-PhCHO at -80 °C in THF/DMF (3:1). p-R-PhCHO's would be considered more

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Table 1. Rate constants and activation enthalpy and entropy values for the deformation of PPA by metal-dioxygen complexes.

<table>
<thead>
<tr>
<th>Comp</th>
<th>k_{0}(M^{-1}s^{-1})</th>
<th>ΔH^‡(kJmol^{-1})</th>
<th>ΔS^‡(JKmol^{-1})</th>
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<td>(-90 to -60)</td>
<td>(-90 to -60)</td>
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<tr>
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<td>(0 to +20)</td>
<td>(0 to +20)</td>
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<td>0.04</td>
<td>(+5 to +35)</td>
<td>(+5 to +35)</td>
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electrophilic than PPA or CCA, therefore these observations were unexpected. Metal-peroxide complexes previously found to be reactive towards PPA or CCA showed higher reaction rates towards \( p-R\text{-PhCHO} \)[1][4-6] By comparing the aldehyde substrates that were reactive towards 1 (PPA/CCA) and those that were poorly reactive (\( p-R\text{-PhCHO} \)’s), we posited that the \( \alpha\text{-H} \) or -C atoms in the aldehyde substrates play a critical role. We investigated the role of the \( \alpha\text{-H} \) atom by determining the \( k_{\text{obs}} \)-value for the reaction between 1 and \( \alpha\text{-deutero-cyclohexanecarboxaldehyde} (\alpha\text{-D-CCA}) \)[7]-[9]. No difference in rate constants for \( \alpha\text{-H}-\text{CCA} \) and \( \alpha\text{-D-CCA} \) was observed. This would suggest that no acid-base or HAA process involving the \( \alpha\text{-H} \) atom occurred in the deformylation reaction. We then reacted 1 with aldehyde substrates with varying degrees of \( \alpha\text{-carbon} \) substitution. No reaction was observed upon addition of 100 equivalents of acetalddehyde (EtA, primary \( \alpha\text{-carbon} \)) or propanal (PrA, secondary \( \alpha\text{-carbon} \)) to 1 at \(-80^\circ\text{C} \) in THF/DMF (3:1). As discussed above PPA/CCA (tertiary \( \alpha\text{-carbon} \)) were reactive towards 1. Finally, we reacted with 100 equivalents of trimethylacetaldheyde (TMA, quaternary \( \alpha\text{-carbon} \)) at \(-80^\circ\text{C} \) in THF/DMF (3:1) (\( k = 0.41 \text{ M}^{-1}\text{ s}^{-1} \), Figures S13 and S14). In summary, the rates of aldehyde degradtion by 1 were controlled by the degree of substitution of the \( \alpha\text{-C}-\text{atom} \), but not by the availability of an \( \alpha\text{-H} \) atom in the aldehyde substrate. The degree of \( \alpha\text{-C} \)-substitution controls how electron-rich the \( \alpha\text{-C} \)-atom is. All of the more electron-rich \( \alpha\text{-C} \)-substrates, PPA, CCA and TMA, were reactive towards 1. All of the electron-poor aldehyde substrates (\( p-R\text{-PhCHO}, \text{EtA, PrA} \)) showed poor reactivity. In contrast, electron poor \( p-R\text{-PhCOCI} \) substrates were very reactive towards 1. We put these contrasting observations down to one of the following: different reaction mechanisms exist for acyl chloride and aldehyde substrates; or the rate-determining step is not influenced by the electrophilicity of the \( \alpha\text{-C} \)-atom; or both. We propose mechanisms for the reactions between 1 and acyl chloride or aldehyde substrates (Schemes 2 and S1) based on mechanisms proposed for metal-peroxo Baeyer-Villiger oxidations.""


[16] At higher [p-R-PhCHO], a slow decrease in intensity of the λmax = 627 nm feature of I over time was observed, indicating I was reacting with p-R-PhCHO at high [p-R-PhCHO]. However, at high [p-R-PhCHO] the substrates displayed poor miscibility with the solvent, preventing accurate kinetic analysis.


Nucleophilic Reactivity of a Copper(II)-Superoxide Complex

**Nucleophilic Superoxide.** A metal-superoxide complex has been found to be a highly reactive nucleophile at low temperatures (-80 °C). The copper(II)-superoxide complex reacts readily with certain electrophiles and is capable of the nucleophilic deformylation of electron-rich aldehydes (Baeyer-Villiger oxidation). These observations provide experimental support for the postulated nucleophilic reactivity of metal-superoxide intermediates in the catalytic cycles of certain nonheme iron enzymes.