Superoxide Dismutase Activity Enabled by a Redox-Active Ligand rather than Metal

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Abstract: Reactive oxygen species are integral to many physiological processes. Although their roles are still being elucidated, they seem to be linked to a variety of disorders and may represent promising drug targets. Mimics of superoxide dismutases (SODs), which catalyze the decomposition of $O_2^{\bullet-}$ to H_2O_2 and O_2, have traditionally used redox-active metals, which are toxic outside of a tightly coordinating ligand. Purely organic antioxidants have also been investigated but generally require stoichiometric, rather than catalytic, doses. Here, we show that a complex of the redox-inactive metal zinc(II) with a hexadentate ligand containing a redox-active quinol can catalytically degrade superoxide, as demonstrated by both reactivity assays and stopped-flow kinetics studies of direct reactions with $O_2^{\bullet-}$ and the zinc(II) complex. The observed SOD catalysis has an important advantage over previously reported work in that it is hastened, rather than impeded, by the presence of phosphate, the concentration of which is high under physiological conditions.

The over-accumulation of reactive oxygen species (ROS) has been linked to seemingly myriad cardiovascular, neurological, and inflammatory disorders as well as certain types of cancer.(1-5) High concentrations of ROS are known to damage biomolecules such as proteins and lipids. Although the roles of ROS in these disorders are still matters of intense speculation, the judicious administration of antioxidants could potentially either halt disease progression or at least alleviate many of the symptoms.(6)

One attractive therapeutic strategy would be to administer a pharmaceutical capable of catalytically degrading one or more sorts of ROS. Over the past several decades, small molecules have been developed to mimic superoxide dismutases (SOD), which catalyze the decomposition of $O_2^{\bullet-}$ to the less toxic H_2O_2 and O_2 (Eq 1).(6-13) The overwhelming majority of these compounds contain manganese. This metal is attractive since it is found in the active sites of mitochondrial SOD(14) and tends to be less toxic than other *redox-active* transition metal ions.(15) Despite these benefits, non-peptide bound manganese is harmful, and its use in humans or animals requires a tightly coordinating ligand.(16) This is exacerbated by the fact that Mn(II) complexes are generally less stable than their analogs with most other divalent transition metal ions.(17) Purely organic antioxidants tend not to be as effective and are usually required in stoichiometric, rather than catalytic, doses. Redox-inactive metals can promote SOD activity by coordinating multiple anions of superoxide; the clustering of $O_2^{\bullet-}$ ions around the positively charged metal ions facilitates their

reaction with each other.(18) The rates of these reactions, however, are second-order in $O_2^{\cdot-}$ and are thereby negligible except at extremely high concentrations of $O_2^{\cdot-}$.

Eq 1) $2\ O_2^{\cdot-} + 2\ H^+ \rightarrow O_2 + H_2O_2$

In the current work, we have complexed a redox-active organic molecule to redox-inactive Zn(II). For the organic component, we used the hexadentate ligand *N*-(2,5-dihydroxybenzyl)-*N,N′,N′*-tris(2-pyridinylmethyl)-1,2-ethanediamine (H_2qtp1). This ligand was previously developed for a redox-responsive contrast agent for magnetic resonance imaging: [Mn(H_2qtp1)(MeCN)](OTf)$_2$ (**1**).(19)

Results

Synthesis and Characterization of the Zinc(II)-Containing Superoxide Dismutase Mimic

Mixing H_2qtp1 and zinc(II) triflate in anaerobic acetonitrile (MeCN) provides [Zn(H_2qtp1)(OTf)](OTf) (**2**) in 90% yield (OTf^- = triflate). The colorless product can be crystallized directly from the reaction mixture by adding ether and cooling the solution. Nuclear magnetic resonance (NMR), mass spectrometry (MS), and elemental analysis were used to corroborate the identity and purity of the product (supplementary Figures 1-3).

The crystal structure of **2** is significantly different from that of the dication from **1** in that (A) the metal center is hexacoordinate Zn(II), rather than heptacoordinate Mn(II), and (B) the quinol does not directly bind to the metal (Figure 1a). The overall geometry is best described as a distorted octahedron, with the coordination sphere consisting of the five N atoms from the H_2qtp1 ligand and one O atom from one of the OTf^- anions. The quinol in the crystal structure is fully protonated, as evidenced by the C−O bond lengths, which average 1.38 Å.(20) ^{19}F NMR analysis of a solution of **2** in D_2O indicates that the OTf^- does not remain coordinated to the Zn(II) in aqueous solution (supplementary Figure 4). When the crystallization is performed at room temperature, crystals of [Zn(H_2qtp1)(MeCN)](OTf)$_2$ (**3**) are instead obtained (supplementary Figure 6). The Zn(II) remains hexacoordinate with the coordination consisting entirely of N-donors. The ability to obtain two crystal structures demonstrates that other molecules and anions can access the metal center in spite of the potential hexadenticity of the H_2qtp1 ligand.(19)

*Characterization of Complex **2** in the Aqueous Phase*

Potentiometric pH titration data suggest that the quinol readily deprotonates to $Hqtp1^-$ and binds to the Zn(II) in aqueous solutions (supplementary Figure 7). The solution structure therefore differs substantially from the solid-state structures obtained from MeCN. The H_2qtp1 ligand by itself was found to undergo two ionization events between pH 2.5 and 9.0. The calculated pK_a values of 7.24 and 4.72 are consistent with the protonation of one of the tertiary amines (H_3qtp1^+) and one of the pyridine rings (H_4qtp1^{2+}), respectively.(17) We were unable to satisfactorily fit the data to models with additional protonation events. The crystal structure of the neutral ligand shows a hydrogen bond interaction between the quinolic OH group and one of the amines, providing a possible explanation for why both tertiary amines are not protonated as the pH is lowered (supplementary Figure 8). Complex **2**, conversely, displays ionization events with pK_a values of 3.17 and 6.20. Our best fits to these data suggest that free ligand is not present to an appreciable degree between pH 2.5 and 9.0; this prevented us from calculating accurate formation constants for either the $[Zn(Hqtp1)]^+$ or $[Zn(H_2qtp1)]^{2+}$ complexes. The pK_a value of 6.20 for the Zn(II) complex is consistent with the (de)protonation of a M(II)-bound phenolate(21, 22) and suggests that the relevant species between pH 7.0 and 8.5 is $[Zn(Hqtp1)]^+$ (Figure 1b). We speculate that the ionization event centered around pH 3.17 corresponds to the (de)protonation of a Zn(II)-bound pyridine group.

The assignment of the 6.20 pK_a value to the (de)protonation of a metal-bound quinol is supported by spectrophotometric pH titration data (supplementary Figure 9). Above pH 7, there is a strong peak at 310 nm; the energy of this is consistent for a intraligand electronic transition for a phenolate, rather than a phenol.(21, 23) As the pH drops below 6, this band disappears and is replaced by a prominent shoulder at 290 nm, an energy that is more consistent with a protonated phenol.

Aqueous solutions of **2** were found to be stable to O_2, and no oxidation was observed by optical spectroscopy over 12 h. Complex **2** was studied by cyclic voltammetry (CV) in an aqueous phosphate solution buffered to pH 7.2. Only a single reversible feature is observed, with an $E_{1/2}$ of 110 mV vs. Ag/AgCl and a ΔE of 95 mV (supplementary Figure 10). The $E_{1/2}$ corresponds to 305 mV vs. NHE, which is close to the theoretically ideal value for the dismutation of $O_2^{\bullet-}$.(12, 24) A similar feature with only a 5 mV greater $E_{1/2}$ was observed for **1**.(19)

*Antioxidant Activity of Complex **2***

The antioxidant activity of complex **2** was tested with three common assays (Figure 2). The ability of the Zn(II) complex to mimic SOD was initially assessed using an established procedure that uses xanthine oxidase to produce $O_2^{\cdot-}$ and a subsequent reaction with lucigenin to detect it.(13, 25) The concentration at which half of the sensing reaction was blocked (IC_{50}) was found to be 16.8 nM. This is equal within error to the 11.3 nM (±10.1) value measured for **1**.(19) Based on the IC_{50} value of 16.8 nM obtained from the lucigenin assay and the 7.7×10^5 M^{-1} s^{-1} rate constant reported for the reaction between $O_2^{\cdot-}$ and lucigenin,(26) one would estimate a k_{cat} of 2×10^8 M^{-1} s^{-1} from these data. We also determined the SOD activity of **2** with a variant of the first assay that instead uses the reaction between $O_2^{\cdot-}$ and cytochrome c as a means of detection (supplementary Figure 12). The cytochrome c assay has the advantage of being much less sensitive to fast radical-radical cross-reactions. The results from the second series of measurements yielded a higher IC_{50} value of 2.52 (±0.44) μM and a lower k_{cat} of 1.06 (±0.20) $\times 10^6$ M^{-1} s^{-1}.

The DPPH assay (DPPH = 2,2-diphenyl-1-picrylhydrazyl radical hydrate) gauges the ability of a compound to donate hydrogen atoms to DPPH to yield its non-radical hydrazine form.(27-29) By this measure, the H_2qtp1 ligand and its Mn(II) and Zn(II) complexes are approximately equivalent hydrogen atom donors (Figure 2). The IC_{50} value for **1** was previously found to be 6.6 μM;(19) this is identical within error to the 7.5 and 8.1 μM values measured for **2** and the metal-free H_2qtp1 ligand. The H_2qtp1 ligand and both of its M(II) complexes are all better antioxidants than ascorbic acid.

The first assay used to assess SOD activity is markedly imperfect, and concerns have been raised about side reactions between the various components influencing the measured IC_{50} and providing a misleading account of the antioxidant activity.(11, 30-33) The errors are also quite high, as the reported IC_{50} value for **1** illustrates. For these reasons, we performed an extensive kinetic analysis of the reactions between $O_2^{\cdot-}$ and **2** in aqueous solutions buffered to pH 7.4 (supplementary Figure 12) and 8.1 (Figure 3). The H_2qtp1 ligand by itself was tested but was not found to catalyze the decomposition of $O_2^{\cdot-}$ at either pH 7.4 or 8.1. The uncatalyzed degradation of superoxide is second-order in $O_2^{\cdot-}$, demonstrating that all potentially catalytic metal impurities have been removed from our aqueous solutions by treatment through a Chelex exchange matrix. All reactions involving the Zn(II) complex, conversely, were found to be first-order with respect to both $O_2^{\cdot-}$ and **2**. The k_{cat} for the reactions run at 25 °C in 60 mM HEPES solutions buffered to

pH 7.4 and 8.1 were 3.4×10^6 M^{-1} s^{-1} and 4.7×10^6 M^{-1} s^{-1}, respectively. These values, while low relative to those of many recently characterized SOD mimics,(10, 34-37) are approximately ten-fold greater than those measured for a recently characterized Mn(II) complex that was found to degrade $O_2^{\cdot-}$ through an outer-sphere mechanism.(38) The k_{cat} value measured at pH 7.4 is still approximately an order of magnitude greater than the rate of the uncatalyzed reaction under those conditions, demonstrating that **2** is indeed serving as a catalyst. The $O_2^{\cdot-}$ (present in at least ten-fold excess over **2**) is completely consumed through first-order decay whenever **2** is present as a catalyst. Since 2×10^{-7} M of **2** is sufficient to catalytically degrade 2×10^{-4} M of $O_2^{\cdot-}$, we estimate that the catalyst can turnover at least 1000 times.

Unexpectedly, the reactions run in 50 mM phosphate solutions buffered to pH 7.4 are faster, corresponding to a k_{cat} of 1.9×10^7 M^{-1} s^{-1} (supplementary Figure 13). The use of phosphate as the buffer had previously been found to invariably slow the rate of manganese-catalyzed superoxide degradation.(10, 11, 38) That the presence of phosphate does not appear to destabilize or otherwise inhibit **2** is noteworthy since intracellular concentrations of phosphate are high.(39, 40)

We confirmed that complex **2** directly reacts with $O_2^{\cdot-}$, using UV/vis spectroscopy and MS. The decreased intensity of the UV/vis band at ~320 nm is consistent with the oxidation of the quinolate portion of the ligand (supplementary Figure 14).(19, 23) Large and physiologically unrealistic excesses of KO_2 appear to degrade the complex. When MS is used to analyze mixtures of KO_2 and **2** over 15 min, we see peaks that are consistent with the oxidation of the methylene bridge between the amine and quinol/*para*-quinone portions of the H_2qtp1 ligand (supplementary Figure 15). At 5 min, we observe a m/z feature that is consistent with the methylene having been oxidized to an amide. At 15 min, we observe a m/z feature that is consistent with the hydrolysis of that amide and the loss of the *para*-quinone moiety. The H_2O_2 product was confirmed qualitatively using peroxide strips.

Discussion

The synthesis of the Zn(II) complex **2** is straightforward, and pure product can be obtained with relatively little effort. The metal center is coordinatively flexible. $[Zn(H_2qtp1)(MeCN)]^{2+}$ and $[Zn(H_2qtp1)(OTf)]^{+}$ cations are observed in crystals obtained from MeCN solution, but the aqueous solution data are more consistent with $[Zn(Hqtp1)]^{+}$, which features a deprotonated quinol

directly coordinating to the Zn(II). The binding affinities of quinols for metal ions increases substantially upon their deprotonation.(23, 41)

Despite the lack of a redox-active transition metal, complex **2** is a competent mimic of superoxide dismutase (SOD). The k_{cat} value of 2×10^8 M^{-1} s^{-1} from the lucigenin assay is two orders of magnitude greater than the 1.06×10^6 M^{-1} s^{-1} value obtained from the cytochrome c assay. The stopped-flow measurements, however, agree with the slower rate constant. The results of the three rate measurements suggest that the products of lucigenin reduction by superoxide are indeed introducing other modes of reactivity into the first assay. Under the reaction conditions, lucigenin cation radicals are generated;(30, 33) these could react with potential quinoxyl radical intermediates and artificially raise the k_{cat}. The discrepancy between the lucigenin assay and the two other measures of SOD activity may therefore be consistent with the formation of a ligand radical during the catalytic cycle.

Based on the k_{cat} values from the aforementioned experiments, we hypothesize that the reactivity with $O_2^{\cdot-}$ could proceed through a Zn(II) complex with a quinoxyl radical-containing ligand. In order to assess the feasibility of such a species, we reacted **2** with $AgSbF_6$ and Et_3N in MeCN. The Ag^+ and Et_3N abstract electrons and protons, respectively. The addition of the $AgSbF_6$ to $[Zn(Hqtp1)]^+$ triggers a brief color change to a darker yellow that persists for about 30 s before turning pink (supplementary Figure 16). A rapidly filtered and freeze-quenched aliquot of a similar reaction run in H_2O taken at 30 s displays feature with a *g* value of 1.99 when imaged by EPR (supplementary Figure 17). Over time, this EPR feature loses intensity, suggesting that it corresponds to an organic radical and not to residual Ag(0). MS analysis of the reaction in MeCN reveals a mixture of Zn(II) complexes with $Hqtp1^-$ and the *para*-quinone-containing qtp1 (supplementary Figure 18). The $[Zn(qtp1)]^{2+}$-containing products are proposed to result from two sequential one-electron oxidations.

Based on our observations, we propose that complex **2** catalyzes the reduction/oxidation of $O_2^{\cdot-}$ through the inner-sphere mechanism illustrated in Figure 4. In the SOD mimicry of small molecule manganese complexes, phosphate is believed to compete with $O_2^{\cdot-}$ for coordination sites on the metal center;(11) this competition leads to lower k_{cat} values for superoxide degradation in phosphate buffer. Strong phosphate binding to some manganese SOD mimetics can even cause ligand dissociation with concomitant formation of manganese phosphate, which itself is SOD active but possesses a smaller k_{cat}.(42) Phosphate is larger than $O_2^{\cdot-}$, and we believe that steric

effects are hindering its ability to coordinate to the Zn(II). Zn(II) is smaller than Mn(II) and has a stronger preference for hexacoordination.(43) These factors, in conjunction with the bulk and hexadenticity of the Hqtp1$^-$ ligand, should greatly hinder the ability of phosphate to coordinate to the metal and thereby inhibit the reactivity with $O_2^{\cdot-}$. Even if some portion of the phosphate gets coordinated and opens some of the chelate rings, this will not influence the catalysis as long as the quinol moiety is coordinated and superoxide binding is not inhibited. This differs from other metal-based catalysis, e.g. by manganese SOD mimetics, where phosphate binding can also affect k_{cat} through modification of the redox potential of the metal center.

The larger k_{cat} for superoxide degradation in phosphate solutions suggests that the buffer component is instead accelerating the reaction. The major phosphate species from pH 7.0-8.5 are $H_2PO_4^-$ and HPO_4^{2-}. The negative charges of these ions allow them to react readily with the positively charged Zn(II) complexes. Only one of the major HEPES species, conversely, has an overall negative charge, and we believe that their lessened attraction to the Zn(II) complexes makes HEPES less efficient as a proton transfer agent. We propose that $H_2PO_4^-$ is donating protons to the coordinated peroxo ligands that would result from the inner-sphere reduction of $O_2^{\cdot-}$ at the metal center. We have MS evidence for what we believe is the Zn(II)-OOH product of this proton transfer (supplementary Figure 19); this would represent strong evidence for the inner-sphere mechanism shown in Figure 4. Unfortunately, we were unable to cleanly generate a ^{18}O-labeled species using $K^{18}O_2$; we believe that the basic impurities present in the KO_2 destabilize the Zn(II)-OOH species.(44) Additional protonation of the HO_2^- to H_2O_2 would aid in its release and allow coordination of the next equiv. of $O_2^{\cdot-}$. Protonating a coordinated $O_2^{\cdot-}$ would facilitate its reduction by the quinolate portion of the ligand by shifting its redox potential to a more positive value. HPO_4^- can also deprotonate the distal OH group of the quinol and lower the potential for its oxidation to a quinoxyl radical. The dual function of phosphate buffer, with involvement of both $H_2PO_4^-$ and HPO_4^{2-} in the catalysis as the proton donor and acceptor, respectively, is supported by pH-dependent measurements of k_{cat} taken at a constant ionic strength of 130 mM (supplementary Figure 20). These demonstrate that the highest SOD activity of **2** (k_{cat} = 2.6 (±0.1) × 10^7 M^{-1} s^{-1}) occurs at pH 7.21. This corresponds to the pK_a of $H_2PO_4^-$, and equal amounts of $H_2PO_4^-$ and HPO_4^{2-} are present in solution under such conditions.

The Zn(II) is proposed to serve three different roles in the catalysis. First, the metal ion lowers the pK_a value of the quinol, rendering it more susceptible to oxidation. Second, coordination

of quinoxyl radical and *para*-quinone to the Zn(II) should also increase their oxidation potentials and facilitate their reduction, with concomitant oxidation of $O_2^{\cdot-}$.(45, 46) Third, the positive charge of the metal ion attracts $O_2^{\cdot-}$ and puts it in close proximity to its redox partners on the ligand.

A large enough excess of $O_2^{\cdot-}$ will degrade the catalyst, and under such circumstances, we see oxidation of the ligand by 5 min (supplementary Figure 15). We speculate that the produced H_2O_2 may be stoichiometrically oxidizing the ligand after the $O_2^{\cdot-}$ is depleted. However, the accumulation of enough H_2O_2 to decompose the catalyst is physiologically unlikely, due to the presence of catalase.

In summary, we prepared a Zn(II) analog of a previously characterized Mn(II) complex with a redox-active ligand. The redox-activity of the ligand allows its Zn(II) complex to behave as a functional mimic of SOD enzymes, which instead rely on redox-active metal ions for their catalysis. We speculate that the metal cation amplifies the intrinsic antioxidant activity of the organic component by attracting $O_2^{\cdot-}$ and facilitating the oxidation and reduction of both redox partners. Using Zn(II) in place of neurotoxic and more weakly bound Mn(II) has the added benefit of rendering the catalysis less sensitive to biologically prevalent phosphate.

Methods

Analysis of the Antioxidant Properties of the Zn(II) Complex

We first tested H_2qtp1 and [Zn(H_2qtp1)(OTf)](OTf) (**2**) using a technique commonly used to measure an antioxidant's ability to degrade superoxide.(25) Superoxide was generated *in situ* from a reaction between xanthine and xanthine oxidase. A subsequent reaction between $O_2^{\cdot-}$ and lucigenin provides a spectroscopic signal that can be used to quantify an antioxidant's ability to degrade $O_2^{\cdot-}$. The copper/zinc superoxide dismutase isolated from bovine erythrocytes (0.001-100 U/ml, Calbiochem) served as a positive control. Each assay was carried out in a total volume of 1 mL containing 50 mM tris (pH 8.0), hypoxanthine (50 μM), xanthine oxidase (0.005 U/ml, Calbiochem) and dark-adapted lucigenin (5 μM) in the presence of either **2** (0.1 nM – 10 μM) or its vehicle. Reactions were carried out at room temperature and were initiated by adding xanthine oxidase to the hypoxanthine-containing solution. Luminescence was measured using a TD-20/20 (Turner Designs) luminometer and expressed as relative light units (RLU). Luminescence was measured for four 10 s integrations after an initial delay of 3 s. The four RLU values were averaged,

and each concentration was expressed as a percent of that produced in the presence of vehicle. Each assay data point was performed in duplicate and each assay was repeated three times.

In order to verify the lucigenin-based *in vitro* assay, the cytochrome c assay was also performed. A reaction between xanthine and xanthine oxidase was again used to generate superoxide, which then reduces cytochrome c in either the presence or absence of **2**. The rate constants for superoxide degradation by **2**, which competes with the reduction of 10 μM cytochrome c by superoxide ($k_{cyt\ c} = 2.6 \times 10^5\ M^{-1}\ s^{-1}$), were determined using time-resolved UV/vis spectroscopy. A Hewlett-Packard Diode Array UV/vis device (model 8452A) was used for the UV/vis experiments. The data were recorded with the OLIS global work program. A mixture of xanthine (0.1 mM, predissolved in concentrated NaOH solution), ethylenediaminetetraacetic acid (0.1 mM) and cytochrome c(III) (10 μM, from bovine heart 95%) in 50 mM tris buffer at pH 8.0 was prepared. Each assay was carried out at room temperature in a total volume of 1 mL in a Quartz Suprasil precision cell (Hellma) in the presence of **2** (50 nM – 40 μM). The reactions were initiated by the addition of xanthine oxidase (0.0017 U/mL). The increase in absorption at 550 nm, which is proportional to the reduction of ferricytochrome c, was measured every 5 s over 2 min. Every measured rate v_i was compared to v_0, the rate of the reactions without **2**. The IC_{50} value represents the concentration of **2** at which v_i equals the half of v_0. Each measurement was performed in duplicate, and the entire assay was repeated three times.

The antioxidant properties of H_2qtp1 and **2** were also assessed using the DPPH assay.(28) Aqueous solutions of either **1**, **2**, or ascorbic acid were added to a solution of 0.10 mM DPPH in MeOH, such that the final reaction volume was 0.2 mL. Samples were incubated in the dark for 30 min at room temperature. Spectrophotometric measurements were subsequently performed at 517 nm using a Molecular Devices Spectramax Plus. This wavelength corresponds to the λ_{max} of the reduced product. Experiments were performed in triplicate and repeated twice.

Determination of in vitro *SOD activity via stopped-flow technique*

Superoxide dismutase activity was tested by a direct method using stopped-flow technique as described elsewhere.(11) Experiments were carried out on a Biologic SFM-400 instrument, using syringes 1, 2 and 3, equipped with an Energetiq LDLS ENQ EQ-99-FC laser driven light source and a J&M TIDAS diode array detector (integration time 0.5 ms, λ = 180 – 724 nm). The source of superoxide was commercially available KO_2 dissolved in dry DMSO ($[O_2^{\cdot-}] \approx 1 – 2$ mM). Each complex was tested at four different concentrations between 0.9 and 9 μM in aqueous solutions

buffered with HEPES or sodium phosphate to either pH 7.4 or pH 8.1. The ionic strength was set to 111 mM unless stated otherwise. The aqueous solution containing the Zn(II) complex was mixed in a 9:1 ratio with the superoxide solution in DMSO using a high-density mixer. In each experiment, the concentration of superoxide exceeded that of the Zn(II) complex by at least ten-fold to ensure catalytic conditions. Millipore water was used for the preparation of the buffer solutions; the buffers were treated with Chelex 100 sodium exchange resin for at least 12 h before use in order to remove adventitious metal ions. The data analysis was performed using the BioKine V4.66 software. Each reported k_{obs} value is the average of at least 10 measurements. k_{cat} values were determined from the slope of the k_{obs} vs. [SODm] plot.

Crystallographic data for the structures reported in this Article have been deposited at the Cambridge Crystallographic Data Centre, under deposition numbers CCDC 1830122 ([ZnH$_2$qtp1](MeCN)](OTf)$_2$), 1830123 ([Zn(H$_2$qtp1)(OTf)](OTf)), and 1830124 (H$_2$qtp1). Copies of the data can be obtained free of charge from www.ccdc.cam.ac.uk/structures/. All other data supporting the findings of this study are available within the Article and its Supplementary Information, and/or from the corresponding authors upon reasonable request

Supplementary Information. Experimental section, spectroscopic data for **2**, structural data for H$_2$qtp2, **2**, and [Zn(H$_2$qtp1)(MeCN)](OTf)$_2$, CV of **2**, results of cytochrome c assay, sample kinetic traces for the decomposition of $O_2^{\cdot-}$, spectroscopic and MS analyses of the reactions between **2** and either $O_2^{\cdot-}$ or Ag^+/base.

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Notes

The authors declare no competing financial interest.

Author Contribution

M. B. W. and A. S. contributed equally to the work. M. B. W. prepared and characterized the complex and analyzed its catalytic activity using the lucigenin and DPPH assays and spectroscopy. M. Y. first prepared the complex and did the preliminary characterization. A. S. performed and interpreted the stopped-flow kinetics, interpreted the data obtained by UHR-CSI-MS and contributed to the formulation of the proposed mechanism. L.S. performed the UHR-CSI-MS measurements. A.S.Z. did the cytochrome c assay. J. D. G. collected and analyzed crystallographic data. D. D. S. assisted with the DPPH and lucigenin assays. I. I.-B. directed the work of A. S., L.S. and A. S. Z., interpreted the data, formulated the proposed mechanism, and wrote part of the manuscript. C. R. G. directed the work of M. B. W. and M. Y., interpreted the data, and was the chief author of the manuscript.

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Figure Captions

Figure 1. Structures of complex **2** obtained from solid-state and aqueous measurements. a) Structure of $[Zn(H_2qtp1)(OTf)]^+$. All hydrogen atoms and the non-coordinated triflate anion are omitted for clarity. All thermal ellipsoids are drawn at 50% probability. Further details about the structure and those for $[Zn(H_2qtp1)(MeCN)](OTf)_2$ are provided in the Supplementary Information. b) Proposed aqueous structure for **2**: $[Zn(Hqtp1)]^+$, where $Hqtp1^-$ is the singly deprotonated form of the H_2qtp1 ligand.

Figure 2. Assays of the antioxidant capabilities of **2**. a) Superoxide scavenging effect of **2**. Superoxide was generated using a hypoxanthine-xanthine oxidase reaction and detected using the chemiluminescent probe lucigenin. Reactions were carried out in 50 mM Tris-HCl (pH 8.0) containing **2**. Data for the various concentrations of **2** are expressed as a percentage of luminescence in the presence of vehicle. b) DPPH free radical scavenging assay of H_2qtp1, **2**, and ascorbic acid. The antioxidants were added to DPPH and incubated in the dark for 30 min at room temperature. Spectroscopic measurements were performed at 517 nm. The data were normalized to the absorbance in the presence of vehicle. Error bars represent the standard deviation of two experiments (n = 2) performed in triplicate.

Figure 3. Kinetic traces of superoxide decomposition. The decreased absorption at 250 nm (60 mM HEPES buffer, pH 8.1) demonstrates that **2** is reacting directly and catalytically with $O_2^{\cdot -}$. Inset: Determination of k_{cat} by linear regression of k_{obs} values, obtained by first-order fits of kinetic traces, vs. [**2**]. In the absence of **2**, the decomposition of superoxide is second-order with respect to $O_2^{\cdot -}$.

Figure 4. Possible mechanistic pathway for the reduction/oxidation of $O_2^{\cdot -}$ by **2**. The reactivity is proposed to proceed through a Zn(II)-ligand radical species. The phosphate ions are proposed to transfer protons to and from the metal complexes.