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A Pro-Chelator Triggered by Hydrogen Peroxide Inhibits Iron-Promoted Hydroxyl Radical Formation

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Neurodegenerative diseases such as Parkinson's and Alzheimer's diseases show signs of increased oxidative stress that result when reactive oxygen species (ROS) overwhelm a cell's inherent antioxidant mechanisms.^{1–4} Markers of oxidative stress include lipid peroxidation, DNA base hydroxylation, and protein modification, all of which are attributed to the highly reactive hydroxyl radical, OH*. While many potential antioxidant therapies use radical scavengers in attempts to mitigate cellular damage, such strategies do not inhibit formation of these harmful radicals.

A principal mechanism for the formation of OH^{\bullet} is via iron-promoted reactions like the Fenton reaction (eq 1),⁵ which becomes catalytic if cellular reductants can reduce Fe^{3+} to Fe^{2+} .

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + OH^{-}$$
 (1)

In order for iron to promote Fenton chemistry, it must be in a coordination environment that favors redox cycling and allows reactants access to the inner sphere of the metal center.⁶ These requirements imply that loosely bound iron that is not properly regulated by the cell's normal metal trafficking and storage mechanisms contributes to oxidative stress. 1,2,4 Chelating agents that can selectively sequester this pool of iron could potentially inhibit iron-promoted oxidative stress by inactivating the source itself. Although several chelators that were developed to treat iron overload diseases have some desirable properties for treating neurodegenerative diseases,⁷⁻¹⁰ they also have troubling drawbacks. 11 Their high affinity for iron means that they compete with iron-binding proteins, thereby altering healthy iron distribution and inhibiting essential iron-containing enzymes. Furthermore, their intrinsic affinity for other metal ions disrupts the availability of key elements like zinc.

To overcome these limitations, we are developing a class of prochelators that have little to no affinity for metal ions until a protective mask is selectively removed by ROS, as demonstrated in Scheme 1 for H_2O_2 . In the absence of oxidative stress, these masked molecules are poor ligands that cannot alter healthy metal ion distribution, a common toxicity issue associated with currently available chelation therapies. Disease conditions that elevate oxidative stress, however, activate the chelator to reveal a high-affinity ligand that scavenges and incapacitates redox-active iron that is the source of OH^{\bullet} generation.

Herein we present our first-generation pro-chelator, **BSIH**, in which a boronic ester conceals a latent phenolic oxygen that is a key donor atom of salicylaldehyde isonicotinoyl hydrazone (**SIH**), a member of the well-known aroylhydrazone class of chelators that show considerable promise as orally available agents.^{12–14}

Aryl boronic esters react selectively with H_2O_2 to produce phenols, 15,16 which are excellent metal-binding groups often incorporated into multidentate ligands. Boronate-based fluoresceins have been used as intracellular fluorescence probes of H_2O_2 , work

Scheme 1

that demonstrates the selectivity and biocompatibility of this class of molecules. 15,16

The pro-chelate **BSIH** is readily obtained by condensation of isonicotinic acid hydrazide with (2-formylphenyl)boronic acid pinacol ester (**Bsal**). The X-ray structure in Figure 1 reveals an E configuration about the C7=N3 double bond and an anti configuration of the B atom with respect to the imine N3 atom. NMR reveals that other conformations are accessible in solution (Supporting Information). Reaction with H_2O_2 converts **BSIH** cleanly to **SIH**, as confirmed by NMR. The OH group of **SIH** adopts a syn conformation that is favorably disposed for tridentate metal chelation via the carbonyl O, the imine N, and the deprotonated phenolate $O^{-.17}$

As shown in Figure 2, addition of Fe^{3+} to a solution of **BSIH** introduces a shoulder in the UV-vis spectrum at 380 nm which is attributed to $Fe(NO_3)_3$ and a slight decrease at 300 nm which may indicate a weak interaction. The absence of new features suggests that a tight Fe^{3+} complex does not form. The addition of H_2O_2 to this mixture, however, results in a new spectrum matching that of $[Fe(\textbf{SIH})_2]^+$, or $[Fe(\textbf{SIH})]^{2+}$ at low ligand/metal ratios. These two species, which form immediately upon mixing, are distinguishable by a ligand-field band at 658 nm that is more pronounced in the mono species than the bis (Figure 2 inset).

Figure 3 shows the time course for iron complex formation following addition of excess H_2O_2 to a methanol solution of $Fe(NO_3)_3$ and **BSIH**. Although the kinetics are complicated by the mono to bis $Fe(SIH)_x$ coordination, the data fit a pseudo-first-order

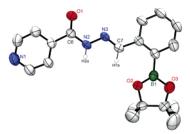


Figure 1. ORTEP diagram of **BSIH** showing 50% thermal ellipsoids. Selected bond distances: C6-O1, 1.228(3); N2-N3, 1.384(2); N3-C7, 1.276(3) Å.

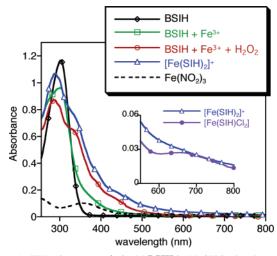


Figure 2. UV-vis spectra of 60 µM BSIH in MeOH in the absence and presence of 30 µM Fe(NO₃)₃. Addition of 0.6 mM H₂O₂ results in a spectrum (open red circles) matching that of $[Fe(SIH)_2]^+$ (blue triangles). The expanded view in the inset compares the mono and bis species, [Fe(SIH)Cl₂(CH₃OH)] and [Fe(SIH)₂]NO₃, respectively, at 60 μ M.

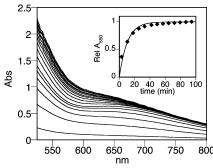


Figure 3. UV-vis spectra showing the formation of $[Fe(SIH)]^{2+}$ and [Fe(SIH)₂]⁺ upon addition of 100 mM H₂O₂ to a solution of 1.5 mM Fe(NO₃)₃ and 3.0 mM BSIH in MeOH.

expression to give $k_{\rm obs} = 1.6 \times 10^{-3} \, \rm s^{-1}$. This value is consistent with preliminary kinetic data for the conversion of BSIH to SIH in the absence of iron (not shown), indicating that the rate-limiting step for iron sequestration is oxidation of BSIH to SIH, followed by rapid metal complexation.

To test the effectiveness of **BSIH** for inhibiting OH• formation, we used an in vitro deoxyribose assay in which hydroxyl radicals that are generated via typical Fenton conditions of Fe³⁺, ascorbic acid, and H2O2 degrade deoxyribose to give products that form a chromophore with thiobarbituric acid (TBA) with λ_{max} at 532 nm. ¹⁸ Figure 4 displays the effect of increasing chelator concentration on the degradation of deoxyribose under these conditions. Values of A/A_0 above 1 indicate that the additive promotes OH $^{\bullet}$ formation, whereas values below 1 indicate that the additive either scavenges OH more efficiently than deoxyribose, or that it inhibits ironcatalyzed OH formation via effective iron chelation. EDTA, a ligand known to promote Fenton chemistry, causes a significant increase in A/A_0 (Supporting Information), whereas desferrioxamine (**DFO**) and **SIH**, chelators known to inhibit Fenton chemistry, ¹⁹ show a decrease in A/A_o. As shown in Figure 4, BSIH protects against deoxyribose degradation as well as both DFO and SIH.

To show that the protective effect of BSIH is not solely due to consumption of H₂O₂, we tested the boronate-masked salicylaldehyde, Bsal, which converts to salicylaldehyde (Sal) in the presence of H₂O₂. Neither Bsal nor Sal has a significant influence on the

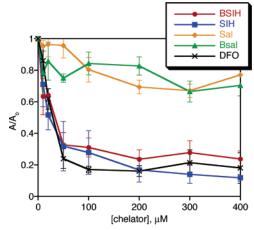


Figure 4. Effect of chelator concentration on deoxyribose degradation by OH $^{\bullet}$. A and A_0 are the absorbance at 532 nm in the presence and absence of chelator, respectively. Values below $A/A_0 = 1$ indicate protection of deoxyribose. Conditions: 200 µM H₂O₂, 10 µM FeCl₃, 2 mM ascorbic acid, 15 mM deoxyribose in pH 7.4 NaHPO₄ buffer.

deoxyribose assay, as shown by the nearly constant A/A_0 values near unity in Figure 4. Whereas **DFO** and **SIH** protect deoxyribose when OH• are generated in the absence of added H₂O₂, **BSIH** has little effect under these conditions (Supporting Information). Taken together, these data indicate that the protective effect of BSIH against deoxyribose degradation derives from its H2O2-dependent conversion to SIH, which in turn provides the right coordination environment around Fe to prevent iron-promoted OH[•] generation.

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Supporting Information Available: Complete refs 9 and 10, experimental details, and X-ray crystallographic data, including CIF files. This material is available free of charge via the Internet at http:// pubs.acs.org.

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