Inorganic Chemistry

Communication

pubs.acs.org/IC

Oxidation of Biologically Relevant Chalcogenones and Their Cu(I) Complexes: Insight into Selenium and Sulfur Antioxidant Activity

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Supporting Information

ABSTRACT: Hydroxyl radical damage to DNA causes disease, and sulfur and selenium antioxidant coordination to hydroxyl-radical-generating Cu^+ is one mechanism for their observed DNA damage prevention. To determine how copper binding results in antioxidant activity, biologically relevant selone and thione ligands and Cu^+ complexes of the formula $[Tpm^*Cu(L)]^+$ [Tpm* = tris(3,5-dimethylpyrazolyl)methane; L = *N*,*N*′-dimethylimidazole selone or thione] were treated with H_2O_2 and the products analyzed by ^1H, $^{13}C\{^1H\}$, and $^{77}Se\{^1H\}$ NMR spectroscopy, mass spectrometry, and X-ray crystallography. Upon H_2O_2 treatment, selone and thione binding to Cu^+ prevents oxidation to Cu^{2+}; instead, the chalcogenone ligand is oxidized. Thus, copper coordination by sulfur and selenium compounds can provide targeted sacrificial antioxidant activity.

Oxidative DNA damage is an underlying cause of diabetes, cancer, and neurodegenerative diseases.[1,2] DNA-damaging hydroxyl radical ($^{\bullet}OH$, reaction 1) forms when Cu(I) reduces hydrogen peroxide, and this copper-mediated DNA damage results in cell death and disease.[3]

$$Cu^+ + H_2O_2 \rightarrow Cu^{2+} + HO^{\bullet} + OH^- \quad (1)$$

Selenium and sulfur antioxidants are well investigated for disease prevention,[4,5] although their mechanisms of action are not fully understood. Two major clinical trials (NPC and SELECT) showed conflicting results for selenium supplementation prevention of prostate cancer, emphasizing a critical need to understand selenium antioxidant mechanisms.[6,7] Our previous work determined that metal coordination is required for inhibition of copper-mediated DNA damage by sulfur and selenium compounds, and this copper-binding mechanism is distinct from traditional mechanisms such as radical-scavenging or glutathione peroxidase-like activity.[8,9]

To determine how coordination to sulfur and selenium inhibits copper-mediated oxidative damage, the reactivity of H_2O_2 with biologically relevant tris(3,5-dimethylpyrazolyl)-methane (Tpm*) copper(I) complexes[10] with *N*,*N*′-dimethylimidazole selone (dmise) and thione (dmit) ligands (Figure 1) is reported. These chalcogenone ligands resemble selenoneine and ergothioneine antioxidants in animals and plants.[11,12] Selenium and sulfur binding to Cu^+ may prevent copper-mediated DNA damage via two routes: (1) coordination of the selone or thione ligand alters the $Cu^{2+/+}$ reduction potential to prevent copper redox cycling, or (2) the bound chalcogenone may oxidize more readily than Cu^+ to act as a *targeted* sacrificial antioxidant. This work investigates H_2O_2 oxidation of dmise and dmit and their Cu^+ complexes, $[Tpm^*Cu(dmise/dmit)]^+$. Elucidating selenium and sulfur DNA damage prevention mechanisms will enable effective antioxidant selection for animal and clinical studies of disease prevention.

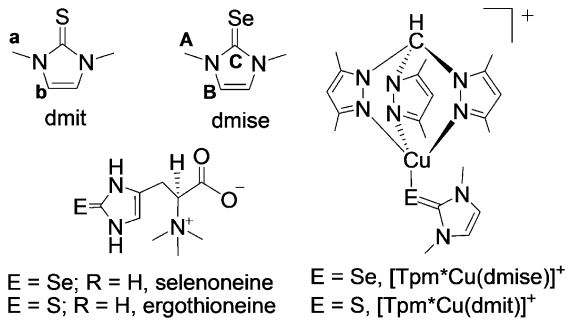

Figure 1. Structures of dmit and dmise with ^1H and $^{13}C\{^1H\}$ NMR resonance labels (lower case and upper case letters, respectively), their Cu^+ complexes, and naturally occurring chalcogenones.

Upon treatment of dmise and dmit with aqueous H_2O_2 (30% w/w), ^1H NMR spectra show shifted resonances corresponding to the methyl protons (δ 3.98 for both) and olefinic protons (δ 7.60 and 7.61 for dmise and dmit, respectively) along with the emergence of a new resonance at δ 8.91 (Figures 2A and S1A in the Supporting Information, SI; resonance labels in Figure 1). $^{13}C\{^1H\}$ NMR resonances of these oxidation products show little shift in the methyl and olefinic carbon resonances relative to the unoxidized chalcogenones. In contrast, the chalcogenone C═Se/S carbon resonance shifts upfield by about δ 20 (Figure S2 in the SI). This upfield shift, coupled with the new ^1H NMR resonance at δ 8.91, indicates cleavage of the C═Se or C═S bond and formation of the dimethylimidazolium cation.[13] The dmise ligand has a $^{77}Se\{^1H\}$ NMR resonance at δ −29.5 that shifts to δ 1345 upon treatment with H_2O_2 (Figure 3), indicating formation of SeO_2 or a similar species.[14] Because fewer equivalents of H_2O_2 are required for complete oxidation, dmise is more prone to oxidation than its sulfur analogue, dmit.

Electrospray ionization mass spectrometry (ESI-MS) on the dmise and dmit oxidation products confirm formation of the dimethylimidazolium cation (m/z 97.07). Two oxidized

Received: June 4, 2013

   dx.doi.org/10.1021/ic401366c | *Inorg. Chem.* XXXX, XXX, XXX−XXX

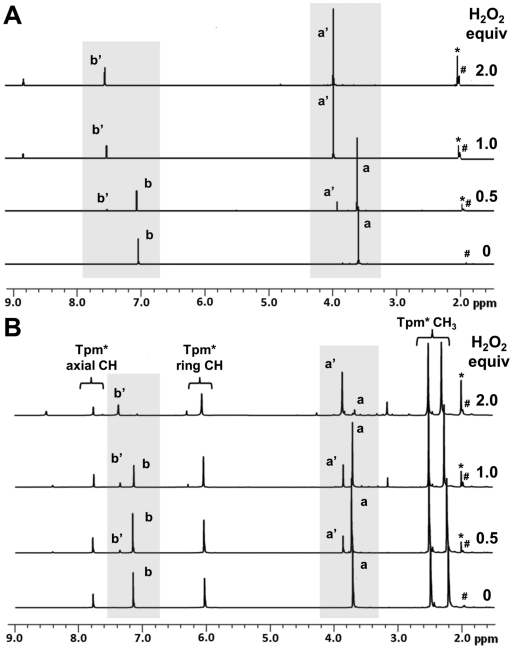

Figure 2. ^{1}H NMR spectra in CD_3CN for (A) dmise and (B) [Tpm*Cu(dmise)][BF_4] upon H_2O_2 treatment. The resonance labeling scheme is given in Figure 1, with the prime symbol indicating resonances arising from oxidation. Residual acetonitrile and H_2O resonances are labeled with a pound symbol and an asterisk, respectively.

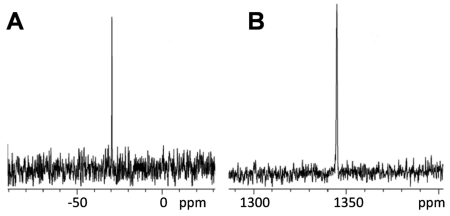

Figure 3. ^{77}Se{^{1}H} NMR spectra of uncoordinated (A) dmise alone and (B) dmise after reaction with 1 equiv of H_2O_2.

selenium products at m/z 112.96, assigned as $[SeO_2H]^-$, and m/z 142.98 are also observed after addition of 1 or 2 equiv of H_2O_2, indicating that the SeO_2-derived species may react with H_2O_2 more readily than dmise. Likewise, two oxidized sulfur products at m/z 79.98 for $[SO_3]^-$ and m/z 96.99 for $[SO_4H]^-$ were identified upon addition of 2 and 3 equiv of H_2O_2 to dmit. In both cases, the resulting sulfur and selenium species are oxidized by more than 1 equivalent of H_2O_2. Bhabak and Mugesh[13] determined that dmise and dmit oxidation by peroxynitrite yields dimethylimidazolium cation and selenium and sulfur oxides, respectively, consistent with H_2O_2 oxidation results.

To determine whether the Cu^+ or chalcogenone ligand preferentially reacts with H_2O_2, acetonitrile solutions of the [Tpm*Cu(dmise/dmit)]$^+$ complexes were treated with up to 2 equiv of H_2O_2 for the dmise complex or up to 3 equiv of H_2O_2 for the dmit complex. ^{1}H NMR spectra of the oxidized Cu(dmise) complex (Figure 2B) show sharply defined peaks even after addition of 2 equiv of H_2O_2, indicating that the diamagnetic Cu^+ center is *not* oxidized upon treatment with H_2O_2!

Upon H_2O_2 oxidation of [Tpm*Cu(dmise)]$^+$, the ^{1}H NMR spectra show shifted resonances at δ 3.86 for the dmise methyl protons, δ 7.36 for the dmise olefinic protons, and a new resonance at δ 8.49 corresponding to one proton (Figure 2B). Tpm* resonances do not shift upon H_2O_2 addition. Similar shifts in the ^{1}H NMR resonances are observed for the oxidized [Tpm*Cu(dmit)]$^+$ complex at δ 3.85 and 7.36, and a new resonance appears at δ 8.47 (Figure S1B in the SI). This increased aromaticity, coupled with the appearance of a new resonance at δ 8.48, indicates formation of the *N*,*N*′-dimethylimidazolium cation. As measured by ^{1}H NMR integration, oxidation reactions for the Cu^+(dmise) and -(dmit) complexes are 85% and 68% complete after addition of 2 and 3 equiv of H_2O_2, respectively.

Resonances for the dimethylimidazolium cation appear in the ^{13}C{^{1}H} NMR spectrum of [Tpm*Cu(dmit)]$^+$ after oxidation at δ 36.3, 124.4, and 137.2. For the [Tpm*Cu(dmise)]$^+$ complex, the methyl resonances for dmise and imidazolium overlap, but the new imidazolium resonance at δ 124.4 is observed, as are copper-bound acetonitrile resonances at δ −7.1 and 111.8.[15] Similar to the ^{1}H NMR spectra, Tpm* resonances of both complexes do not shift significantly upon H_2O_2 addition. Despite acquisition times of up to 24 h, no ^{77}Se NMR signals were observable for these oxidation reactions.

Mass spectrometry data for the oxidized products obtained from the treatment of [Tpm*Cu(dmise/dmit)]$^+$ with H_2O_2 indicate formation of [Tpm*Cu($NCCH_3$)]$^+$ (m/z 402.12) and *N*,*N*′-dimethylimidazolium (m/z 97.07), corroborating ^{1}H NMR results. No signals attributable to Cu^{2+} species were observed in these mass spectra. Negative-ion ESI-MS results indicate the same oxidized sulfur ($[SO_3H]^-$ and $[SO_4H]^-$) and selenium ($[SeO_2H]^-$) products observed upon oxidation of dmise and dmit. Similar to the chalcogenones alone, the dmise ligand in [Tpm*Cu(dmise)]$^+$ is more sensitive to H_2O_2 oxidation than dmit in [Tpm*Cu(dmit)]$^+$.

The products of these oxidation studies are in direct contrast to O_2 oxidation of [Tpm*Cu($NCCH_3$)]$^+$ that forms an hydroxo-bridged complex, $[\{Tpm^*Cu(OH)\}_2]^{2+}$, with concomitant oxidation of both Cu^+ centers to Cu^{2+}.[16] Oxidation of [Tpm*Cu($NCCH_3$)]$^+$ with 2 equiv of H_2O_2 results in a blue-green solution with extremely broad ^{1}H NMR resonances, and formation of Cu^{2+} species was confirmed by mass spectrometry. Treatment of metal thiolate complexes of iron, nickel, platinum, and ruthenium with H_2O_2 or O_2 results in formation of sulfinate and sulfonate complexes with no change in metal oxidation state.[17−21] In contrast, thioether complexes treated with O_2 or H_2O_2 form sulfinate and sulfenate species only with Cu^{2+} species or with concomitant oxidation of Cu^+ to Cu^{2+}.[22,23] Thus, the metal-bound selone or thione ligand enables targeted, sacrificial H_2O_2 scavenging to prevent Cu^+ oxidation (Figure 4A).

Examination of the highest occupied molecular orbitals (HOMOs) of the [Tpm*Cu(L)]$^+$ (L = dmise/dmit/$NCCH_3$) complexes from the DFT (mPW1PW91)-optimized geometries is consistent with protection of Cu^+ by the chalcogenone ligands. The HOMOs for the dmise and dmit complexes have significant S/Se p character, but the HOMO of the acetonitrile complex is centered on the metal (Figure 4C). Thus, the redox-active chalcogenone ligands will be preferentially oxidized, but inert ligands will allow oxidation of the metal. The bound chalcogenone molecular orbitals are also destabilized relative to the free ligand, suggesting that coordination enhances their ability to scavenge reactive oxygen species.

The acetonitrile complex [Tpm*Cu($NCCH_3$)][BF_4] (Figure 4) was isolated from oxidation of [Tpm*Cu(dmise)]$^+$. Although Cu^+ acetonitrile complexes with tris(pyrazolyl)-containing ligands are reported,[24] and this acetonitrile complex has been independently synthesized,[25] its structure has not been elucidated. The Cu^+ in [Tpm*Cu($NCCH_3$)][BF_4] adopts

dx.doi.org/10.1021/ic401366c | *Inorg. Chem.* XXXX, XXX, XXX−XXX

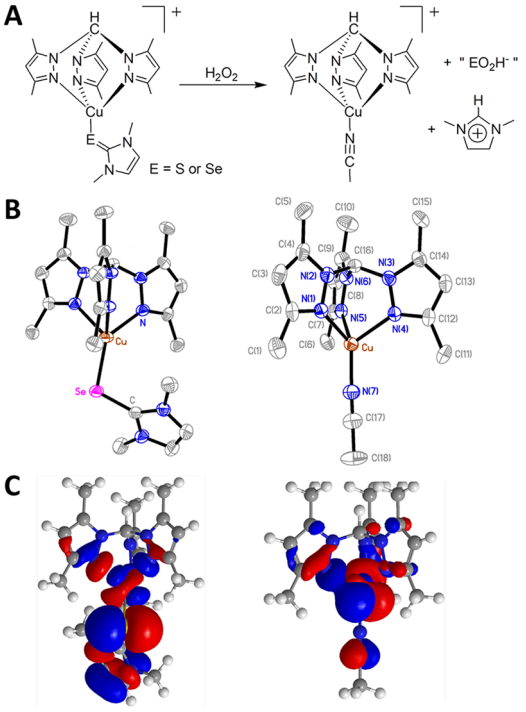

Figure 4. (A) Reaction of $[Tpm^*Cu(L)]^+$ (L = dmise or dmit) with H_2O_2. (B) X-ray crystal structures of $[Tpm^*Cu(dmise)]^+$ (left, from ref 10) and $[Tpm^*Cu(NCCH_3]^+$ (right, isolated from a reaction mixture; 30% ellipsoids; anions and hydrogen atoms removed for clarity). (C) HOMOs for $[Tpm^*Cu(dmit)]^+$ (left) and $[Tpm^*Cu(NCCH_3)]^+$ (right).

distorted tetrahedral geometry, bound in a κ^3 fashion to three nitrogen atoms from the Tpm* ligand and terminally bound to acetonitrile. The N−Cu−N angles range from 85.9 to 89.9°, with Cu−N bond lengths of 2.08−2.09 Å (Table S1 in the SI), comparable to similar tris(pyrazolyl)methane copper(I) complexes.[25,26] The Cu−N bond distance of 1.87 Å for the terminal acetonitrile bond is comparable with the reported $Tp^{CF_3,CH_3}Cu(NCCH_3)$ complex.[15]

Dmise and dmit oxidation by H_2O_2, alone or in $[Tpm^*Cu(dmise/dmit)]^+$ complexes, results in oxidation of the Se and S atoms, cleavage of the C═Se or C═S bond, and dimethylimidazolium cation formation. When bound to Cu^+, the selone and thione ligands protect Cu^+ from oxidation. Therefore, copper-mediated damage may be prevented in vivo by coordination to sulfur and selenium compounds. This mechanism of chemoprotection could be an important target for the treatment of diseases caused by oxidative stress.

■ ASSOCIATED CONTENT

Supporting Information

X-ray crystallographic data in CIF format, experimental details, ^1H and $^{13}C\{^1H\}$ NMR spectra of dmise and dmit titrations with H_2O_2, and details of DFT calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

J.L.B. and C.A.B. thank the NSF for support (Grants CHE 0545138 and 0750413, respectively). M.M.K. thanks the Clemson University Chemistry Department for a fellowship and Carolyn Quarles for performing the ESI-MS experiments.

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dx.doi.org/10.1021/ic401366c | *Inorg. Chem.* XXXX, XXX, XXX−XXX