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# Cu(II)-catalyzed oxidation of thiols by superoxide ligated to Co<sup>III</sup>

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Copper(II) dramatically catalyzes the oxidation of thiols by a superoxide bridging two Co<sup>III</sup> ions. The catalyzed path overwhelmingly dominates over the uncatalysed path and is first order in the superoxo complex concentration. The first-order rate constants show a first-order dependence in  $[Cu^{2+}]$ , a second-order dependence in [thiol] and linearly varies with  $[H^+]^{-3}$ . On the basis of observed kinetics reported here, it is proposed that Cu(II) reacts with two thiol molecules to form a Cu<sup>II</sup>(thiol)<sub>2</sub> complex, an electron is transferred from one ligated thiol to the Cu<sup>II</sup> center to form Cu<sup>I</sup>(thiol) and a thiyl radical. The copper(I)-thiol complex is oxidized by the conjugate base of the title complex to regenerate Cu<sup>II</sup>(thiol). A Cu<sup>II/I</sup> catalytic cycle is thus believed to be responsible for the observed catalysis. Copyright © 2012 John Wiley & Sons, Ltd.

Supporting information may be found in the online version of this article.

Keywords: superoxide; 2-mercaptoethanol; thioglycolic acid; L-cysteine; cobalt(III); kinetics

# INTRODUCTION

Aliphatic thiols are vulnerable to oxidation by many oxidizing agents and lead to disulphides, sulphoxide, and so forth, depending on the thermodynamic strength of the oxidant.<sup>[1-6]</sup> The thioredoxins are small proteins that demonstrate a wide range of redox activity in plants and animals, all involving their two redox reversible sulfhydryl groups. In biological systems, thiols are oxidized by flavins, cytochromes, and dehydroascorbic acid to control the cellular redox potential and prevent oxidative damage.<sup>[7–9]</sup> Often trace metal ions, especially Cu<sup>2+</sup> ion, catalyze such reactions.<sup>[10,11]</sup> Even ubiquitous Cu<sup>2+</sup> ions present as impurities in the solution affect the kinetics.<sup>[12]</sup>

The present work deals with the Cu(II)-catalyzed oxidation of few thiols, *viz* 2-mercaptoethanol, thioglycolic acid, and cysteine (a paradigm for the aliphatic thiols) in aqueous acidic media by superoxide radical ligated to  $\mu$ -amido- $\mu$ -superoxobis[tetraaminecobalt(III)] ion (1). In the presence of externally added Cu<sup>2+</sup> (10–100  $\mu$ M), the catalyzed path dominates overwhelmingly over the uncatalyzed path, kinetics of the latter had been reported earlier.<sup>[13]</sup>

# EXPERIMENTAL

#### Materials

2-Mercaptoethanol (mercap) and thioglycolic acid (tga) (Aldrich, Saint Louis, Missouri, USA) solutions were prepared with 99% reagent grade thiols. L-cysteine (cys) (SRL, India) was dissolved in water to prepare the stock solution. Sodium perchlorate solution for the maintenance of ionic strength in kinetic measurements was prepared from NaHCO<sub>3</sub> and HClO<sub>4</sub>. All other materials used were of reagent grade and used without further purification. Thiols were dissolved just prior to the kinetic studies and fresh solutions were always used. Doubly distilled and then freshly boiled water was used throughout the studies.

The superoxo complex,  $\mu$ -amido- $\mu$ -superoxobis[tetraamminecobalt (III)] tetranitrare (1) was synthesized using a literature process<sup>[14]</sup> and recrystallized from 0.5% HNO<sub>3</sub>. The complex 1 was characterized by elemental analysis, FTIR and Raman Spectroscopy.

#### Instrumentation

Elemental analyses (H, N) of complex **1** were performed on a Perkin-Elmer 240 C elemental analyzer. IR spectra were recorded on a Perkin-Elmer RX1 FTIR spectrum spectrophotometer with the sample prepared as a KBr pellet in the range 4000–400 cm<sup>-1</sup>, and Raman spectroscopy was performed on Scientific NXR FT-Raman MODULE, Thermoelectron Corporation, USA, Nd:YVO<sub>4</sub> laser with 1064 nm excitation. Absorbance and UV-vis spectra were recorded with a Jasco V-650 spectrophotometer (Jasco International Co. Ltd., Hachioji, Tokyo, Japan) using 1.00 cm quartz cells. The kinetics was monitored *in situ* in an electrically controlled thermostated ( $25.0 \pm 0.1$  °C) cell housing. Acid solutions were standardized by pH metric titration using a Metrohm 736 GP Titrino autotitrator (Metrohm AG, Switzerland).

#### **Kinetic measurements**

Kinetics was determined following decrease in absorbance at 700 nm, one of the absorption peaks of the superoxo complex (**1**) at 25.0 °C and at ionic strength, l = 0.50 M (NaClO<sub>4</sub>). Copper(II) ion was added in the concentration range 10–100  $\mu$ M as its perchlorate salt. This amount is in overwhelming excess over the copper impurities measured by Atomic Absorption in the solvent water ( $2.2 - 2.5 \times 10^{-6}$  M). A large excess of perchloric acid and thiol over **1** (0.50 mM) in argon-saturated media was maintained in all the kinetic runs. In the presence of externally added Cu<sup>2+</sup> (much more than present in the reaction media as impurity), the reaction followed excellent first-order kinetics. The observed first-order rate constants ( $k_o$ ) were extracted by nonlinear least-squares fitting of the absorbance ( $A_t$ ) versus time (t) data to a standard first-order exponential decay equation.

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#### Stoichiometry

The complex **1** was allowed to completely react with each of the mercapto derivatives. The unreacted mercap, tga, or cys were then quantified spectrophotometrically<sup>(15,16)</sup> in separate experiments.

# **RESULTS AND DISCUSSION**

#### Characterization of the complex 1

Elemental analysis: *Anal.* Calcd for  $[(NH_3)_4Co(NH_2,O_2)Co(NH_3)_4]$  $(NO_3)_4$ : H, 4.73, N, 33.1. Found: H, 4.63, N, 33.2%. Main IR absorption bands observed for **1** (KBr Pellet/cm<sup>-1</sup>) are 3239 (s) and 2098 (w) for v(NH), 1635 (s), 1386 (s) and 1313 (s) for  $\delta_{as}(NH_3)$  and 825 (s) for  $\rho_r(NH_3)$  match well with the reported<sup>[17]</sup> values for this complex (v, stretching vibration;  $\delta_{as}$ , asymmetric deformation vibration;  $\rho_{rr}$ , rocking vibration). The observed Raman shift of 1074 cm<sup>-1</sup> for the O⊠O stretching also confirms the presence of the superoxo bridge.<sup>[18]</sup> Purity of **1** was also checked by comparing its absorbance at 700 nm ( $\epsilon$  in M<sup>-1</sup> cm<sup>-1</sup>, Found 300; reported 306).<sup>[14]</sup>

#### Stoichiometry and the reaction products

The reaction under the kinetic conditions indicated a consumption ratio,  $\Delta$ [**1**]:  $\Delta$ [thiol] = 1:1 (Table S1). It suggests oxidation



Figure 1. Structure of  $\mu$ -amido- $\mu_1$ -hydroperoxo-bis[tetraamminecobalt (III)]]<sup>4+</sup> ion, **2H** 



**Figure 2.** Time-resolved spectra of 0.50 mM of **1** reacting with 5.0 mM mercap.  $[H]^+=0.2 \text{ M}$ ,  $[Cu^{2+}]=5 \times 10^{-5} \text{ M}$ , I=1.0 M (NaClO<sub>4</sub>),  $T=25.0 \degree \text{C}$ . (a): spectrum of pure complex shown in red; (b)–(q): spectra of reaction mixtures stated above at 120, 300, 420, 600, 780, 960, 1140, 1320, 1500, 1680, 1860, 2100, 2340, 2580, 2820 and 3060 s, respectively

product for all thiols is their disulphides, respectively.<sup>[10,19]</sup> The most common one-electron reduction product of **1** in our reaction condition is the  $\mu$ -amido- $\mu$ -hydroperoxo-bis[tetraamminecobalt(III)]<sup>4+</sup> ion, **2H** (Fig. 1).<sup>[20,21]</sup> A family of spectra for reactions of **1** with cysteine recorded at different time interval is shown in Fig. 2, and the final spectrum is also closely similar in shape and peak positions to those determined for similar  $\mu$ -hydroperoxo binuclear Co(III) complexes with {N<sub>5</sub>O} coordination.<sup>[12,20,21]</sup> A clean conversion of the superoxo complex **1** to the hydroperoxo complex **2H** is therefore anticipated.

The time-resolved spectra of the reaction indicates that the superoxo ligand of the complex **1** undergoes reduction to its peroxo moiety, **2**, and immediately takes up a proton from the solution to be converted into protonated peroxo species **2H** that is more stable in the acidic media.<sup>[22]</sup> The overall plausible reaction sequence is shown below, where the superoxo and peroxo species bear their residual charges and RSH denotes all the three thiols in general.



**Figure 3.** Variation of  $k_o$  with [mercap]<sup>2</sup>. [**1**] = 0.50 mM, [H<sup>+</sup>] = 0.20 M, [Cu<sup>2+</sup>] = 5 × 10<sup>-5</sup> M, *I* = 1.0 M (NaClO<sub>4</sub>), T = 25.0 °C



**Figure 4.** Variation of  $k_o$  with [TGA]<sup>2</sup>. [1] = 0.50 mM, [H<sup>+</sup>] = 0.60 M, [Cu<sup>2+</sup>] = 5 × 10<sup>-5</sup> M, *I* = 1.0 M (NaClO<sub>4</sub>), T = 25.0 °C



**Figure 5.** Variation of  $k_{\rm o}$  with [cys]<sup>2</sup>. [1] = 0.50 mM, [H<sup>+</sup>] = 0.20 M, [Cu<sup>2</sup> +] = 1 × 10<sup>-4</sup> M, *I* = 1.0 M (NaClO<sub>4</sub>), T = 25.0 °C



**Figure 6.** Variation of  $k_0/([Cu^{2+}][mercap]^2)$  with  $1/[H^+]^3$ . [1] = 0.50 mM, [mercap] = 5.0 mM, [Cu<sup>2+</sup>] = 5 × 10<sup>-5</sup> M, /= 1.0 M (NaClO<sub>4</sub>), T = 25.0 °C



**Figure 7.** Variation of  $k_0/([Cu^{2+}][TGA]^2)$  with  $1/[H^+]^3$ . [1] = 0.50 mM, [TGA] = 5.0 mM,  $[Cu^{2+}] = 5 \times 10^{-5}$  M, I = 1.0 M (NaClO<sub>4</sub>), T = 25.0 °C



**Figure 8.** Variation of  $k_o/([Cu^{2+}][cys]^2)$  with  $1/[H^+]^3$ . [1] = 0.50 mM, [cys] = 5.0 mM,  $[Cu^{2+}] = 1 \times 10^{-4}$  M, I = 1.0 M (NaClO<sub>4</sub>), T = 25.0 °C



**Figure 9.** Variation of  $k_0$  with [Cu<sup>2+</sup>]. [1] = 0.50 mM, [mercap] = 5.0 mM, [H<sup>+</sup>] = 0.20 M, *I* = 1.0 M (NaClO<sub>4</sub>), T = 25.0 °C



**Figure 10.** Variation of  $k_o$  with  $[Cu^{2+}]$ . [1] = 0.50 mM, [TGA] = 5.0 mM,  $[H^+]$  = 0.60 M, I = 1.0 M (NaClO<sub>4</sub>), T = 25.0 °C

$$2 \mathbf{1}^{4+} + 2 \text{ RSH} \to 2 \mathbf{2}^{3+} + \text{RSSR} + 2 \text{ H}^+$$
(1)

$$2^{3+} + H^+ \rightarrow 2H^{4+}$$
 (2)

#### **Kinetics and mechanism**

The title complex **1** suffers practically no decomposition in under the reaction conditions. In the presence of excess [thiol] over [**1**] and externally added Cu<sup>2+</sup>, absorbance of **1** reduces with time and the process followed excellent first-order kinetics. A family of time-resolved spectra describing such changes is shown in Fig. 2, and a typical first-order plot is displayed in Fig. S1. The evaluated first-order rate constants,  $k_o$ , showed a square dependence on [thiol] (Figs. 3–5; Tables S2–S4), linear dependence on  $[H^+]^{-3}$  (Figs. 6–8; Tables S2–S4), and a first-order dependence on [Cu<sup>2+</sup>] (Figs. 9–11; Tables S2–S4). To verify if specific medium



**Figure 11.** Variation of  $k_o$  with  $[Cu^{2+}]$ . **[1**] = 0.50 mM, [cys] = 5.0 mM,  $[H^+]$  = 0.20 M, I = 1.0 M (NaClO<sub>4</sub>), T = 25.0 °C

effects has any contribution towards the observed acid dependence in the reaction,<sup>[23]</sup> we have measured first-order rate constants ( $k_o$ ) in presence of LiClO<sub>4</sub> in the entire acidity range (Tables S2–S4). The  $k_o$  values thus found nicely match (within 5%) with the  $k_o$  values obtained when the ionic strength was maintained with NaClO<sub>4</sub>. We thus believe that the observed pH-dependence originates from the properties of the redox partners and not due to any kind of medium effects. From these observations, we propose the reaction sequence shown in Scheme 1.

Deprotonation of the two thiol molecules in presence of Cu<sup>2+</sup> accounts for the two [H<sup>+</sup>]<sup>-2</sup> dependence observation. Another inverse dependence on [H<sup>+</sup>] could only come from 1. We propose dissociation of an H<sup>+</sup> ion from one of the coordinated NH<sub>3</sub> molecules in **1**, thus forming a conjugate base  $[(NH_3)_5Co$  $(O_2)Co(NH_3)_4(NH_2)]^{4+}$  (**1**<sub>-H</sub>) as the exclusive kinetically reactive species(Equation 3).<sup>[24,25]</sup> The superoxo coordinated to **1** has no dissociable proton, and the observed inverse proton dependence could not be traced at the superoxo moiety in 1. Cu(II) reacts with thiols to form [Cu<sup>II</sup>(RS<sup>-</sup>)<sub>2</sub>] complex species (Equations 4 and 5); an electron is transferred from one of the ligated thiolate anions to the Cu<sup>II</sup> center to form [Cu<sup>I</sup>(RS<sup>-</sup>)] and a thiyl radical (Equation 6). The copper(I)-thiol complex thus formed is oxidized by **1** at the rate step to produce  $[Cu^{II}(RS^{-})]^{+}$  that again reacts with another molecule of RSH to regenerate  $[Cu^{II}(RS^{-})_{2}]$ (Equation 5). Two of the thiol radicals condense to form the corresponding disulfide product (Equation 9).

The **Scheme** 1 leads to Equation (10), assuming reaction shown in Equation (6) is fast and quantitatively complete<sup>[26,27]</sup> (see Supporting Information).

$$k_{\rm o} = \frac{k_2 K_1 K_2 K_{\rm -H} \left[ {\rm Cu}^{2+} \right] \left[ {\rm RSH} \right]^2}{\left[ {\rm H}^+ \right]^3} \tag{10}$$

Equation (10) abides all the kinetic dependences with  $k_2K_1K_2K_{-H} =$  2.38 (±0.1) × 10<sup>3</sup> s<sup>-1</sup> for mercap, 9.12 (±0.5) × 10<sup>4</sup> s<sup>-1</sup> for tga

$$I^{4+} \qquad \underbrace{K_{-H}}_{I_{-H}} \qquad I_{-H}^{3+} + H^{+} \qquad (3)$$

$$Cu^{2+} + RSH \xrightarrow{K_1} [Cu^{II}(RS^{-})]^+ + H^+ \qquad (4)$$

$$[Cu^{II}(RS^{-})]^{+} + RSH \xrightarrow{K_{2}} [Cu^{II}(RS^{-})_{2}] + H^{+}$$
(5)

$$[Cu^{II}(RS^{-})_{2}] \xrightarrow{k_{1}} [Cu^{I}(RS^{-})] + RS^{\bullet}$$
(6)

$$1_{-H}^{3^+} + [Cu^{I}(RS^{-})] \xrightarrow{k_2} 2^{2^+} + [Cu^{II}(RS^{-})]^+$$
(7)

$$2^{2^+} + 2H^{+} \xrightarrow{\text{fast}} 2H^{4^+}$$
 (8)

$$2 \text{ RS}^{\bullet} \xrightarrow{\text{fast}} \text{RSSR}$$
(9)

Scheme 1. Reaction Sequence of Cu(II) catalyzed oxidation of thiols by 1

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and 4.44 ( $\pm$ 0.3)  $\times$  10<sup>3</sup> s<sup>-1</sup> for cysteine at 25.0 °C. The values suggest that mercap and cysteine react at comparable rates with **1** in presence of Cu<sup>2+</sup>, but tga reacts at a much faster rate.

The substitution-inert Co<sup>III</sup> centers of the Co<sup>III</sup> superoxo complex (**1**) cannot be reduced by an inner-sphere path. Nevertheless, increased  $[H]^+$  decelerated the rate, a feature unusual for an outer-sphere reaction of a Co<sup>III</sup> complex.<sup>[28-30]</sup> This can be simply explained through equilibria (3), (4), and (5). Deprotonation of **1** (Equation 3) increases its reactivity, whereas metal-assisted deprotonation (Equations 4 and 5) of thiols increases their reactivity. Solutions of Cu<sup>+</sup> in the presence of excess RS<sup>-</sup> result in the formation of [(Cu<sup>1</sup>)(-SR)] complexes.<sup>[31]</sup> In addition, reduction of Cu<sup>III</sup> to Cu<sup>III</sup> by thiols are well-known reactions (Equation 6).<sup>[24,32,33]</sup> Although Cu(I) is not stable in aqueous solution.<sup>[34]</sup> a number cationic and anionic complexes of Cu(I) are found to be stable in aqueous solution.<sup>[35,36]</sup> In fact, complexes of glutathione and Cu(I) have been suggested to be intermediates in cellular metabolism and storage of Cu(I).<sup>[37]</sup>

Thus, the striking catalytic effect exhibited by Cu(II) in the experiments is mainly attributed to the fact that Cu(II) is reduced to the unipositive state Cu(I) by organic thiols under the experimental conditions, and also that the reactivity of Cu(I) is greatly enhanced when bonded with chelating thiol ligands. Thus, the **Scheme** 1 proposed here seems logical for explaining the Cu<sup>2+</sup>-ion catalyzed oxidation of aliphatic thiols by metal-bound superoxide.

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