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An Iron-Based Cytosolic Catalase and Superoxide Dismutase Mimic Complex

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The development of metallodrugs with antioxidant activities is of importance as a way to protect organisms exposed to stressful conditions. Although iron chemistry in the presence of H_2O_2 is usually associated with pro-oxidant activity, mainly via the Fenton reaction, we found that the mononuclear compound $[Fe(HPClNOL)Cl_2]NO_3$ (**1**; $C_{15}H_{18}Cl_3FeN_4O_4$, a = 8.7751(3) Å, b = 9.0778(4) Å, c = 24.3869(10) Å, β = 93.370(2)°, monoclinic, $P2_1/c$, Z = 4), containing the tripodal ligand 1-[bis(pyridin-2-ylmethyl)amino]-3-chloropropan-2-ol, decomposes hydrogen peroxide and superoxide anion in vitro as well as shows in vivo protection because it prevents the harmful effects promoted by H_2O_2 on *Saccharomyces cerevisiae* cells, decreasing the level of lipid peroxidation. This protective effect was observed for wild-type cells, as well as for mutant cells, which do not present the antioxidant metalloenzymes catalase (Ctt1) or copper/zinc superoxide dismutase (Sod1).

Reactive oxygen species (superoxide anion, hydroxyl radical, etc.) and hydrogen peroxide may be generated in the respiratory chain.[1,2] Usually these species are very well managed because aerobic organisms employ different strategies to overcome the deleterious effects of these oxidant species. However, increased levels of these species have been associated with a variety of pathophysiological events such as ischemia, reperfusion-related injuries like heart attack, stroke, and organ dysfunction, as well as neurodegenerative disorders including Alzheimer's, Parkinson's, and Huntington's diseases.[3]

Catalases (CAT) and superoxide dismutases (SOD) are the major antioxidant metalloenzymes involved in the elimination of H_2O_2 and $O_2^{\bullet-}$, respectively. CAT possess an iron(III) protoporphyrin IX or a dinuclear manganese active site.[4] The SOD active site may contain iron, manganese, copper/zinc, or nickel.[5]

Concerning the development of CAT and SOD mimetics, some manganese complexes have shown CAT and/or SOD activity in vitro and/or in vivo.[6−8] On the other hand,

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although iron complexes have been able to decompose H_2O_2/$O_2^{\bullet-}$ in vitro,[9] to the best of our knowledge, there have been no reports to date on iron compounds that can perform in vivo protection against dangerous H_2O_2 effects, acting as both CAT and SOD mimetics, although there are some that have shown protection on cultured cells.[10]

Investigating compounds with biological activity, we have employed the tripodal ligand 1-[bis(pyridin-2-ylmethyl)amino]-3-chloropropan-2-ol (HPClNOL)[11] in the syntheses of dinuclear iron(III) and mononuclear copper(II) and manganese(II) complexes, which showed nuclease, antitumoral, and catalase activities, respectively.[12] Now, we were able to isolate the mononuclear compound $[Fe(HPClNOL)Cl_2]$ NO_3 (**1**), which shows in vitro and in vivo antioxidant activities.

The molecular structure of **1**[13] (Figure 1) reveals that the Fe^{III} ion shows a N_3OCl_2 coordination environment composed of the tripodal ligand HPClNOL and two chorido coligands. The pyridyl groups are trans to each other, and the alcohol group is protonated, as indicated by the long bond length Fe1−O1. Furthermore, its hydrogen atom was found from the Fourier map.

Compound **1** shows a *quasi*-reversible redox process at −88 mV vs Fe/Fe^+,[14] confirming its mononuclear structure in a CH_3CN solution (see Figure 1 in the Supporting Information, SI). This potential is lower than those observed for the complexes containing three pyridine groups, $[Fe(L1)Cl_2]^+$ [$E_{1/2}$ = 97 mV vs $Ag/AgNO_3$, L1 = TPA = tris(2-pyridylmethyl)amine] and $[Fe(L2)Cl_2]^+$ [$E_{1/2}$ = 65 mV vs $Ag/AgNO_3$, L2 = bis(2-pyridylmethyl)(2-pyridylethyl)amine],[15] but higher than those containing two pyridine and one amide or two pyridine and one carboxylate groups, $[Fe(PABMPA)Cl_2]^+$ ($E_{1/2}$ = −222 mV vs Fe/Fe^+)[16] and $Fe^{III}(PBMPA)Cl_2$ ($E_{1/2}$ = −473 mV vs Fe/Fe^+).[9a] This result indicates that the alcohol group present in **1** is a better donor than the pyridine group present in the complexes $[Fe(L1)Cl_2]^+$ and $[Fe(L2)Cl_2]^+$ but a worse donor than the amide and carboxylate groups observed in the compounds $[Fe(PABMPA)Cl_2]^+$ and $Fe^{III}(PBMPA)Cl_2$, respectively.

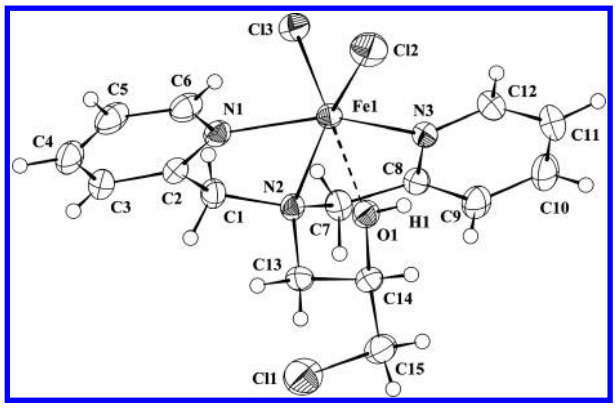

Figure 1. View of the cation **1**. Selected bond distances (Å) and angles (deg): N1−Fe1 2.1345(15), N2−Fe1 2.2034(14), N3−Fe1 2.1191(14), Cl2−Fe1 2.2283(6), Cl3−Fe1 2.2989(5), O1−Fe1 2.1743(12), O1−H1 0.77(2); N1−Fe1−N2 76.00(5), N1−Fe1−N3 154.44(5), N1−Fe1−O1 92.00(5), N1−Fe1−Cl2 100.16(4), N1−Fe1−Cl3 91.10(4), N2−Fe1−N3 78.51(5), N2−Fe1−O1 76.41(5), N2−Fe1−Cl2 163.77(4), N2−Fe1−Cl3 93.29(3), N3−Fe1−O1 83.80(5), N3−Fe1−Cl2 104.86(4), N3−Fe1−Cl3 88.49(4), O1−Fe1−Cl2 88.08(4), O1−Fe1−Cl3 168.16(4), Cl2−Fe1−Cl3 102.61(2). Displacement ellipsoids are drawn at the 50% level.

Volumetric studies showed that **1** produces O_2 in the presence of H_2O_2. Colorimetric experiments to evaluate the nitroblue tetrazolium (NBT) reduction promoted by the $O_2^{\bullet-}$ anion generated by the xantine/xantine oxidase system[17] revealed that **1** was able to avoid the NBT reduction (see Figures 2 and 3 in the SI). These results confirm that **1** can be considered functional CAT and SOD mimetic models in vitro, and they prompt us to investigate whether **1** would be able to promote protection against oxidative stress generated by H_2O_2 in vivo. This study was performed using *Saccharomyces cerevisiae* cells (wild and mutant types) as a eukaryotic cell model.

The data presented in Table 1 show that the wild type (wt) of *S. cerevisiae* cells grown in fermentative metabolism (2% YPD) and treated with **1** showed an acquisition of tolerance against H_2O_2 stress because about 52% of the cells survived to the lethal oxidative stress compared with 7% of the cells without treatment. We used *S. cerevisiae* cells grown in fermentative metabolism because of the hypersensitivity presented by these cells to oxidants. In such conditions (fermentative metabolism), several antioxidant genes are repressed and, thus, proteins like CAT and SOD are not expressed, making it easier to determine whether some compound can act as an antioxidant. A similar protective effect was also observed in respiratory metabolism (4% YPGly). These results confirm our hypothesis that **1** may act as an in vivo antioxidant. The next step was to understand how the protection mechanism works. To access this information, a lipid peroxidation assay was carried out. The results showed that the level of molondialdehyde (MDA), a final product of lipid peroxidation, was reduced to that of the unstressed cells, which can, in fact, be related to an antioxidant property of this compound in reducing the oxidative stress biomarker.

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(13) Crystallographic data for **1**: yellow crystals with parallelepiped shape were isolated from a $MeOH/H_2O$ solution: chemical formula $C_{15}H_{18}Cl_3FeN_4O_4$, fw 480.53 g mol^{-1}, a = 8.7751(3) Å; b = 9.0778(4) Å; c = 24.3869(10) Å; β = 93.370(2)°, space group $P2_1/c$, V = 1939.27(13) $Å^3$, Z = 4, T = 295 K, λ = 0.710 73 Å, D_{calcd} = 1.646 g cm^{-3}, μ = 1.220 mm^{-1}, R1 = 0.0293, wR2 = 0.0772 [$I > 2\sigma(I)$].

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Table 1. Protective Effect of **1** during H_2O_2 Exposure of *S. cerevisiae* Cells[a]

		survival (%)		lipid peroxidation [nmol of MDA (mg of cells)$^{-1}$]	
S. cerevisiae cells	experimental conditions	2% YPD	4% YPGly	2% YPD	4% YPGly
wild type	normal growth	100.0 ± 0.0^a	100.0 ± 0.0^a	0.05 ± 0.010^a	ND
	stressed	7.4 ± 3.0^b	68.0 ± 13.5^b	0.13 ± 0.006^b	ND
	treated and stressed	51.7 ± 2.3^c	100.0 ± 0.0^c	0.06 ± 0.006^a	ND
ctt1△	normal growth	ND	100.0 ± 0.0^a	ND	0.15^a
	stressed	ND	17.6 ± 4.9^b	ND	1.12^b
	treated and stressed	ND	73.2 ± 8.9^c	ND	0.79^c
sod1△	normal growth	100.0 ± 0.0^a	ND	0.07 ± 0.015^a	ND
	stressed	11.0 ± 2.6^b	ND	0.11 ± 0.013^b	ND
	treated and stressed	97.4 ± 4.4^c	ND	0.06 ± 0.019^a	ND

[a] Different letters represent statistically different results when compared to the normal growth in the same group (strain) at $P < 0.05$; ND = not determined. For survival and lipid peroxidation assays, cells were cultured on liquid 2% YPD or 4% YPGly. The media composition determines the kind of yeast metabolism. While 2% YPD is composed of 2% glucose, 2% peptone, and 1% yeast extract, 4% YPGly replaces glucose by glycerol. Glycerol is a nonfermentable source of carbon in which cells grow under respiratory metabolism. Under a high glucose concentration, such as in 2% YPD, yeast cells grow under fermentative metabolism.

In order to have new insight about the mechanism of protection, two mutant strains of *S. cerevisiae* harboring deficiencies in the synthesis of cytoplasmatic enzymes copper/zinc superoxide dismutase (Sod1) and catalase (Ctt1) were studied. Using such mutant cells is a suitable strategy to understanding whether a metal complex might be acting as a mimic of these antioxidant metalloenzymes. Thus, these deficient cells on Sod1 or Ctt1 were treated with **1** and exposed to H_2O_2 under the same conditions as those described for wt.[18]

In both cases, complex **1** increased significantly the survival of mutant cells against exposure to H_2O_2, while the stressed cells, lacking **1** treatment, showed a very low survival level. Furthermore, after incubation with **1**, followed by H_2O_2 stress, the mutant cells showed a significant decrease in the oxidation of lipids when compared with the stressed cells without treatment. It is worth noting that compound **1** showed complete protection against oxidative stress generated by H_2O_2 on the sod1 mutant cell, keeping the survival and lipid peroxidation levels in the same range as those observed to the unstressed cells. These results are intriguing because of their discrepancy with those of another published study,[19] in which the iron exacerbates the oxidative damages (vacuolar fragmentation) on sod1 mutant cells. This suggests that the tripodal HPClNOL ligand is inducing an antioxidant behavior on the iron center, resulting in a compound that was able to protect *S. cerevisiae* cells against oxidative stress.

Taken together, our results demonstrate that **1** is able to protect wt *S. cerevisiae* cells and also those lacking catalase and copper/zinc superoxide dismutase enzymes. Interestingly enough, **1** demonstrates an ability to overcome the absence of two of the main protective factors in *S. cerevisiae* against oxidative stress. To the best of our knowledge, **1** is the first iron compound that has shown in vivo protection against oxidative stress induced by H_2O_2 in cells lacking cytosolic catalase or superoxide dismutase enzymes. Finally, our study highlights a new compound that might function in biological systems as catalase and superoxide dismutase mimics and may be a new prototype for the development of metallopharmaceuticals with antioxidant activity.[20]

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Supporting Information Available: Synthesis of **1**, X-ray data, cyclic voltammetry, volumetric and NBT studies concerning catalase and superoxide dismutase activities, biological investigation assays, and crystallographic data in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org. CCDC 640206 contains the supplementary crystallographic data for this paper. The atomic coordinates for this structure have also been deposited with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, upon request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, U.K.

(18) Because catabolite repression exerted by glucose in the CTT1 gene expression, we measured the survival of Ctt1-deficient cells only in respiratory metabolism. This metalloenzyme is crucial for cells in respiratory metabolism as a protective mechanism against endogenous H_2O_2 production. Contrary to the CTT1 expression, the expression of SOD1 is initially detected in fermentative metabolism. This explains the reason for testing Ctt1- and Sod1-deficient cells in respiratory and fermentative metabolism, respectively.

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(20) It is important to note that neither the in vitro nor the in vivo antioxidant activities shown by compound **1** mean that it should be considered a pharmaceutical. All that the compound shows at this point is biological activity. We thank the reviewer who suggested that we add this note of caution.