Catalase-Like Activity of a Non-Heme Dibenzotetraaza[14]annulene−Fe(III) Complex under Physiological Conditions

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Hydrogen peroxide is an ubiquitous metabolite in living systems, produced at increased levels in a variety of pathological situations. For the detoxification of H_2O_2, and probably also for maintaining its regulatory function, nature has developed a family of highly effective enzymes, the catalases, which dismutate H_2O_2 according to eq 1:[1,2]

$$2\ H_2O_2 \xrightarrow{\text{catalase}} O_2 + 2\ H_2O \qquad (1)$$

In the past there have been attempts to develop complexes that mimic the catalytic action of catalase as models for the study of the molecular mechanisms of H_2O_2 dismutation. Contrary to their low significance in living systems, a large portion of the compounds reported to exhibit "catalase-like" activity are manganese complexes, mainly because of their potential application as therapeutics.[3] Catalase models that were built around iron(III) centers were mostly based on heme-type structures;[4,5] non-heme iron complexes have been much less investigated for their catalase activity or, more general, "oxygen-activation" properties.[4−14] However, although these compounds decompose H_2O_2,[15−16] the notation "catalase mimic" in our view often appears to be rather euphemistic. Most of the published models have inherent serious deficiences with respect to the preferred characteristics of a "good" catalase mimic, that is, water-solubility, quantitative O_2 production obeying the stoichiometry of eq 1, catalytic activity at physiological pH values (pH 7 ± 1) and micromolar catalyst and H_2O_2 concentrations, and low activity as oxygenating (peroxidase-like) agent.[17] We now find that the non-heme iron(III)-tetraaza[14]-annulene complex **1** provides a promising starting point for the development of useful catalase mimics in that it catalyzes, at micromolar concentrations, the release of O_2 in high yield from low concentrations of H_2O_2 in buffer solution at pH 7.2.

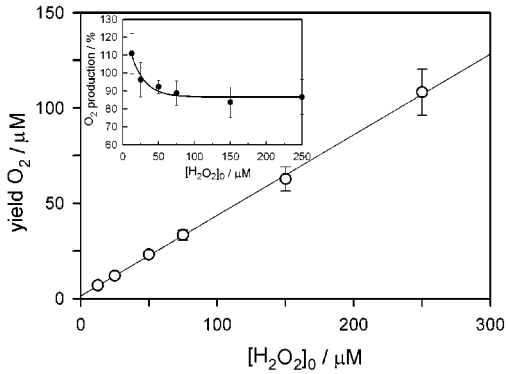
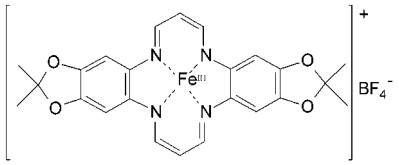


Figure 1. Oxygen release from 12.5 to 250 μM hydrogen peroxide at 25 °C catalyzed by 5 μM **1** at pH 7.2 (50 mM phosphate buffer). Inset: Relative yield (%) of O_2 according to the stoichiometry of eq 1 as a function of H_2O_2 concentration.



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Complex **1**[18,19] is stable in the solid state at room temperature in the absence of air, soluble in DMSO and DMF, and sufficiently soluble in phosphate buffer pH 7.2 containing 0.2−1% DMSO. The UV/vis spectrum in aqueous solution is characterized by three maxima at λ_{max} (log ϵ) = 303 (4.28), 365 (4.16), and 780 (3.44) nm. The two longer-wavelength absorptions are largely diminished after reaction of the complex with H_2O_2. Cyclic voltammetry (3 mM **1** in DMF; 100 mV/s) showed a reversible Fe(III)/Fe(II) redox potential at $E_{1/2}$ = 0.23 V versus Ag/AgCl (∼0.45 vs NHE), close to the potential of $FeCl_3$ under the same conditions ($E_{1/2}$ = 0.19 V vs Ag/AgCl).

The catalase-like activity of **1** was established by electrochemically monitoring (Clark-type electrode) the release of O_2 at 25 °C in 50 mM phosphate buffer pH 7.2.[20] Figure 1 displays the yield of O_2 as a function of the H_2O_2 concentration in the presence of 5 μM **1**.

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(1) For a recent review on the structures and biological functions of catalases see: Zamocky, M.; Koller, F. *Prog. Biophys. Mol. Biol.* **1999**, *72*, 19−66.

(2) The prosthetic group of typical catalases is a heme-type iron(III) (mainly protoporphyrin IX) complex. To date three manganese-centered non-heme catalases are also known, found only in procaryontic cells.

(3) Doctrow, S. R.; Huffman, K.; Marcus, C. B.; Musleh, W.; Bruce, A.; Baudry, M.; Malfroy, B. In *Antioxidants in Disease: Mechanisms and Therapy*; Sies, H., Ed.; Academic Press: San Diego, 1997; pp 247−269.

(4) Robert, A.; Look, B.; Momenteau, M.; Meunier, B. *Inorg. Chem.* **1991**, *30*, 706−711.

(5) Murata, K.; Panicucci, R.; Yopinath, P.; Bruice, T. C. *J. Am. Chem. Soc.* **1990**, *112*, 6072−6083.

(6) Melnyk, A. C.; Kjeldahl, N. K.; Rendina, A. R.; Busch, D. H. *J. Am. Chem. Soc.* **1979**, *101*, 3232−3240.

(7) Cairns, C. J.; Heckman, R. A.; Melnyk, A. C.; Davis, J. M.; Busch, D. H. *J. Chem. Soc., Dalton Trans.* **1987**, 2505−2510.

(8) Zhang, D. L.; Busch, D. H. *Inorg. Chem.* **1994**, *33*, 5138−5143.

(9) Zhang, X. P.; Zhang, D. L.; Busch, D. H.; van Eldik, R. *J. Chem. Soc., Dalton Trans.* **1999**, 2751−2758.

(10) Käpplinger, I.; Keutel, H.; Jäger, E.-G. *Inorg. Chim. Acta* **1999**, *291*, 190−206.

(11) Okuno, T.; Ito, S.; Ohba, S.; Nishida, Y. *J. Chem. Soc., Dalton Trans.* **1997**, 3547−3551.

(12) Than, R.; Schrodt, A.; Westerheide, L.; van Eldik, R.; Krebs, B. *Eur. J. Inorg. Chem.* **1999**, 1537−1543.

(13) Ito, S.; Okuno, T.; Matsushima, H.; Tokii, T.; Nishida, Y. *J. Chem. Soc., Dalton Trans.* **1996**, 4479−4484.

(14) Provent, C.; Chautemps, P.; Gellon, G.; Pierre, J.-L. *Bull. Soc. Chim. Fr.* **1997**, *134*, 487−493.

(15) It is common knowledge that hydrogen peroxide is catalytically decomposed by a variety of "free" and complexed transition metal ions; for a recent overview, see: Salem, I. A.; El-Maazawi, M.; Zaki, A. B. *Int. J. Chem. Kinet.* **2000**, *32*, 643−666.

(16) For rates of Fe(II)-, Fe(III)-catalyzed H_2O_2 decompositions see: Tachiev, G.; Roth, J. A.; Bowers, A. R. *Int. J. Chem. Kinet.* **2000**, *32*, 24−35 and references therein.

(17) Autzen, S.; Korth, H.-G.; Sustmann, R.; de Groot, H., *Eur. J. Org. Chem.* **2001**, 3119−3125.

(18) The preparation of **1** followed the published route for a phenyl-unsubstituted analogue.[19] The structure was confirmed by satisfactory analytical and spectroscopic data. The detailed preparation will be reported in a forthcoming full paper.

(19) (a) de Sloof, G. *Recl. Trav. Chim. Pays-Bas* **1935**, *54*, 995−1010. (b) Hiller, H.; Dimroth, P.; Pfitzner, H. *Liebigs Ann. Chem.* **1968**, *717*, 137−147.

(20) Typically, an aliquot of a 0.6 mM stock solution of **1** in DMSO was added to 1 mL of a solution of hydrogen peroxide in deoxygenated buffer in a DW1/CB1-D3 electrochemical cell (Hansatech) fitted with a Clark-type electrode. After the electrochemical signal had reached a constant level, no further oxygen release (by addition of native catalase) could be monitored, proving that the hydrogen peroxide had been completely destroyed by the catalyst.

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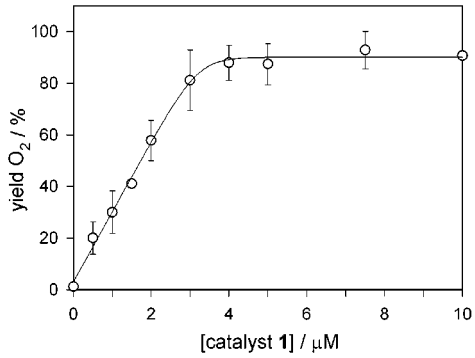

Figure 2. Percentage oxygen production from 250 μM H_2O_2 at 25 °C in 50 mM phosphate buffer pH 7.2 as a function of the concentration of catalyst **1,** with respect to the stoichiometry of eq 1.

The O_2 yield showed an apparent linear dependence on the initial H_2O_2 concentration. The slope (0.434) of the regression line ($r^2 = 0.9993$) is close to the value 0.5 as expected for a catalase-like stoichiometry (eq 1) and, thus, would correspond to an average yield of O_2 of ca. 87%. However, when the percentage yields of O_2 were plotted against the initial H_2O_2 concentration, we observed that below 100 μM H_2O_2 the relative O_2 yield increases with decreasing H_2O_2 concentration to reach about 110 $\pm$ 10% at 12.5 μM H_2O_2 (Figure 1, inset). Such a behavior would indicate the occurrence of a side reaction which eventually leads to an inactivation of the catalyst. The leveling-out at $[H_2O_2] \geq$ 100 μM then would reflect the relative rates of the O_2-producing and the deactivation reactions at high $[H_2O_2]/[\mathbf{1}]$ ratios. At $[H_2O_2]/[\mathbf{1}] \leq 4$ increasing deviation from the catalase stoichiometry is indicated. This view is supported by the dependence of the relative yield of O_2 on the concentration of **1** at a fixed H_2O_2 concentration, as exemplified by Figure 2.

The oxygen yield increases almost linearly with increasing concentration of **1** to reach the constant level of 87 $\pm$ 8% at a point where the ratio $[H_2O_2]/[\mathbf{1}]$ has dropped to 79 $\pm$ 14. This indicates a deactivation of the catalyst after a turnover of about 79 mol of hydrogen peroxide per mol of catalyst. [21]

The formation of O_2 followed clean pseudo-first-order kinetics with half-lives in the range of 30 s or less. The initial rate of oxygen formation (and thus, H_2O_2 decomposition) determined at $[\mathbf{1}] = 2.5$, 5, and 7.5 μM, was linearly dependent on the initial peroxide concentration (Figure 3). The same is true for the dependence of the initial rate on the concentration of **1** (data not shown)**,** indicating second-order kinetics for the rate-determining step. From the data of Figure 3 an apparent rate constant of $k_{app} = 1.5 \times 10^3\ M^{-1}\ s^{-1}$ was calculated for the $H_2O_2 + \mathbf{1}$ reaction. Thus, complex **1** is about 4000 times less efficient than native catalase ($k_{CAT} = 6 \times 10^6\ M^{-1}\ s^{-1}$)[22] but several orders of magnitude faster than "free" Fe(III) or its common amino polycarboxylic acid complexes.[16] The fact that no substrate saturation could be reached in the electrochemically accessible range of H_2O_2 concentrations (O_2 degassing above 500 μM $[H_2O_2]_0$), points to a high K_m value in the mM range (catalase:[21] $K_m = 1.1$ M).

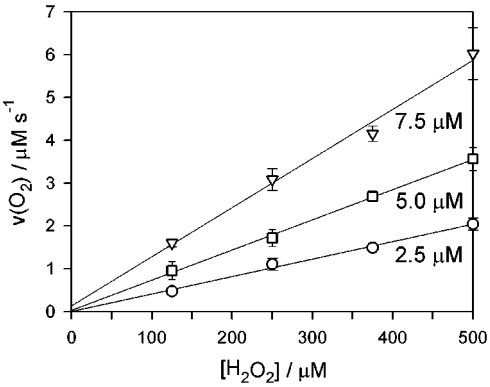

Figure 3. Initial rates of oxygen formation at pH 7.2 and 25 °C as function of the hydrogen peroxide concentration in the presence of 2.5, 5.0, and 7.5 μM complex **1**.

The question remains why complex **1** is more effective in its catalase-like activity under physiological conditions than other non-porphyrin Fe(III) complexes reported thus far.[4,6,9,11−13,17] Apart from pH dependencies this might tentatively be attributed to the presence of the electron-donating alkoxy substituents at the benzene rings. In the catalase cycle of H_2O_2 dismutation, a ferryl iron−porphyrin radical cation species, $Por^{\bullet+}Fe(IV)=O$, plays a central role.[1,23] As a first hypothesis one might argue that the electron-donor properties of the alkoxy substituents in **1** would facilitate a similar formation of a radical cation of the aza[14]-annulene ligand.[24] Experiments to elucidate the mechanism of H_2O_2 dismutation by **1** and to elaborate the influence of other substituents at the benzene positions in the Fe(III)−aza[14]-annulene system to further improve the catalase-like activity are currently underway.

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(21) Preliminary experiments provide strong evidence that the deactivation reaction is initiated by a Fenton-type decomposition of hydrogen peroxide with release of free OH radicals, which then oxidize the annulene ligand of complex **1**. Thus, when a solution of 35 μM **1** in buffer pH 7.25 was reacted with 534 μM H_2O_2 in the presence of the spin-trap compound 5,5-dimethyl-pyrroline-*N*-oxide (DMPO), the characteristic four-line ESR spectrum of the DMPO−OH adduct radical was observed, in addition to DMPO adducts of several carbon-centered radicals. Likewise, after reaction of 396 μM **1** with 5.9 mM H_2O_2 in D_2O solution in the presence of 2.6 mM of [d_6]-DMSO small amounts of deuterated methanesulfonic acid, methanol, and formate were detected by ^{1}H- and ^{13}C NMR spectrometry. These products are very indicative for the intermediacy of free OH radicals, see, e.g.: Richeson, C. E.; Mulder, P.; Bowry, V. W.; Ingold, K. U. *J. Am. Chem. Soc.* **1998**, *120*, 7211−7219.

(22) Bergmeyer, J.; Grassl, M., Eds. *Methods of Enzymatic Analysis*, 3rd ed.; Verlag Chemie: Weinheim, 1983; Vol. II, p 165.

(23) Weiss, R.; Mandon, D.; Wolter, T.; Trautwein, A. X.; Muther, M.; Bill, E.; Gold, A.; Jayaraj, K.; Terner, J. *J. Biol. Inorg. Chem.* **1996**, *1*, 377−383.

(24) For instance, it has recently been shown that the mode of O−O bond cleavage, viz. homolytic vs heterolytic, of intermediate Fe(III)−porphyrin peroxo complexes, which determines the reactivity of Fe(III)−porphyrin/peroxide systems, is governed by the electron density on the ligand: Nam, W.; Han, H. J.; Oh, S. Y.; Lee, Y. J.; Choi, M. H.; Han, S. Y.; Kim, C.; Woo, S. K.; Shin, W. *J. Am. Chem. Soc.* **2000**, *122*, 8677−8684.