Reactions of Tetraazamacrocyclic Fe^{III} Complexes with Hydrogen Peroxide − Putative Catalase Mimics?

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Iron(III) complexes of macrocyclic tetraaza ligands [**Fe(III)-1**–**Fe(III)-3**] were investigated for their putative catalase-like properties under physiological conditions: i.e., in aqueous solution at pH = 7.0–7.4 and at micromolar concentrations of the catalyst and hydrogen peroxide. Complex **Fe(III)-1**, originally studied by Busch et al. as a catalase model, at pH = 4.6, only degrades hydrogen peroxide to oxygen as a minor reaction at this pH (< 1% O_2 yield). Experiments with the analogous complex **Fe(III)-2** at pH = 7.2 found that no oxygen was formed under physiological conditions, although hydrogen peroxide was decomposed to an extent of more than 50%. Complex **Fe(III)-3** produced oxygen on reaction with H_2O_2, but only in stoichiometric amounts. Thus, the decomposition of hydrogen peroxide by these iron(III) complexes cannot reasonably be described as a catalase-like activity.

Introduction

The preparation of synthetic enzymes is a challenge, because low molecular mass model compounds are expected to catalyze the same reaction as the enzyme, hopefully by the same mechanism and with high activity and efficiency. Catalases belong to the most active enzymes and in nature serve to protect living cells from the toxicity of hydrogen peroxide,[1,2] a ubiquitous metabolite produced by various oxidases and superoxide dismutases. Hydrogen peroxide seems to perform regulatory functions at low concentrations, but causes cell damage at higher levels, especially if hydroxyl radicals are generated through interaction with low-valent metal ions such as iron(II).[3] To prevent this damage, cells make use of catalases or peroxidases to neutralize hydrogen peroxide, in a process in which two hydrogen peroxide molecules are transformed into two molecules of water and one molecule of oxygen. Eucaryotic and procaryotic cells mainly use enzymes containing Fe^{III}−porphyrin systems for this purpose. A few manganese complexes are found as active components[3] in catalases from procaryotic cells.

Several catalases containing iron(III)−porphyrin units have been isolated and structurally analyzed by X-ray crystallography,[1] and the mechanism through which hydrogen peroxide is decomposed has been established.[4,5] The primary step is the interaction of one molecule of hydrogen peroxide with the iron(III) center of the porphyrin unit to give a ferryl iron complex (Fe^{IV}=O). As hydrogen peroxide is a two-electron oxidant, a porphyrin radical cation is formed simultaneously with the ferryl species. The catalytic cycle is concluded by reaction between the ferryl iron−porphyrin radical cation (Compound I in enzymologists' terminology) and a second molecule of hydrogen peroxide. Oxygen is liberated and iron(III)−porphyrin is regenerated. The mechanism through which manganese-containing enzymes destroy hydrogen peroxide cannot be the same, since these enzymes contain two manganese ions in their active centers.

Attempts to synthesize both iron and manganese complexes as catalase mimics − that is, compounds that decompose hydrogen peroxide into water and oxygen − have been made. So far, more success seems to have been achieved with organometallic manganese compounds, in particular Mn−salen complexes.[6] It has long been known that hydrogen peroxide is decomposed catalytically in the presence of iron complexes. The chemistry of iron(II)/iron(III) with hydrogen peroxide in aqueous solution is rather complex and the mechanistic details are still debated.[7−11] Less common are studies in which Fe^{III} complexes are viewed as catalase mimics able to decompose hydrogen peroxide efficiently, and perhaps by a mechanism similar to that of the enzyme.[12−14] In 1979, Melnyk et al.[15] introduced a non-heme tetraazamacrocyclic Fe^{III} complex [**Fe(III)-1**], for which oxygen evolution was observed on interacting with hydrogen peroxide under acidic conditions. An account on previous studies of decomposition of hydrogen peroxide by iron(III) compounds is given in that publication. The same group later[16] proposed that this catalytic activity proceeds through a high oxidation state Fe intermediate rather than through involvement of free hydroxyl radicals. The complex therefore had catalase-like and peroxidase-like activity attributed to it. Recently, Busch's group also described a newly designed substituted triazacyclononane−Fe^{II} complex which decomposes hydrogen peroxide by a complex, multistep mechanism.[16,17]

Our goal is the development of low molecular mass Fe^{III} complexes that are soluble in water and decompose hydro-

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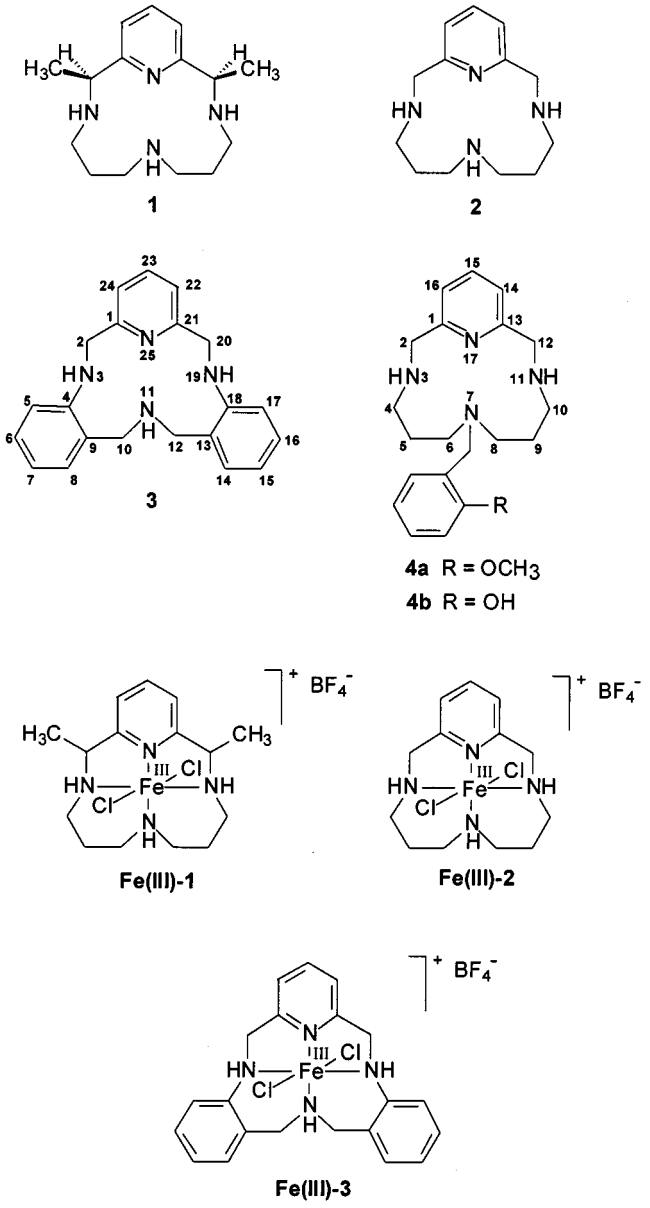

Scheme 1

gen peroxide to oxygen and water in high yield under physiological conditions (i.e. at pH = 7.0−7.4) and at micromolar concentrations of both catalyst and hydrogen peroxide. Such compounds should be attractive for biological applications. Porphyrin systems, on which many model studies have been carried out, were discounted, mainly because of their low solubility in aqueous solution, making them inappropriate for biological investigations. As a starting point for our work we decided to reinvestigate the catalase-like activity of the Fe^{III} complex of **1** [**Fe(III)-1**] under physiological conditions and to progress from this compound to related structures that might be suitable as catalase mimics. Here we report on our investigations on the non-annulene-type tetraazamacrocyclic Fe^{III} complexes **Fe(III)-1**−**Fe(III)-3**.

Results and Discussion

Synthesis of Ligands and Fe^{III} Complexes

Ligands **1** and **2** were prepared according to literature procedures.[16,18] The preparation of ligand **3** was devised similarly, starting from pyridine-2,6-dicarbaldehyde; this was treated with the appropriate diamine in a copper template reaction to give the macrocycle. Diimine reduction by sodium borohydride and removal of copper(II) as sulfide provided **3** in 81% overall yield. In native catalase, a tyrosine residue occupies the distal site of the iron(III)−porphyrin unit. In order to simulate this situation, ligands bearing 2-methoxy or 2-hydroxybenzyl groups at the nitrogen atom opposite to the pyridine nitrogen atom (**4a**, **4b**) were envisaged. The synthesis of these ligands started from 2-methoxybenzylamine, which was added to 2 equiv. of acrylonitrile in a Michael-type reaction. Reduction of the dinitrile to the diamine provided the building block, which could be subjected to macrocyclization as in the case of ligands **1**−**3**.

An iron(III) complex had so far been known only for **1** [**Fe(III)-1**]. This complex was synthesized according to the procedure of Melnyk et al.[15] Compounds **Fe(III)-2** and **Fe(III)-3** were prepared similarly, but with addition of tetraethylammonium tetrafluoroborate instead of tetrafluoroboric acid after air oxidation. Unfortunately, attempts to prepare iron(III) complexes of ligands **4** were unsuccessful, although a number of different experimental conditions were tested.

Reactions of Fe(III)-1−Fe(III)-3 with Hydrogen Peroxide

Rates of reaction of complex **Fe(III)-1** with hydrogen peroxide had been studied thoroughly as functions of the initial concentration of the catalyst (72 μM−1.5 mM), hydrogen peroxide concentration (68−360 mM), and pH (3.94−5.10).[15] Rate constants were obtained from initial rates of oxygen production, determining the formation of oxygen from the pressure increase in a closed reaction system. Unfortunately, Melnyk et al.[15] did not mention the total yield of oxygen formed under their conditions. The only information given is that oxygen production satisfies the stoichiometry implied by the catalase mechanism. We were able to reproduce these results by performing experiments with [H_2O_2] = 260 mM and [**Fe(III)-1**] = 1 mM at pH = 4.6, using a Toepler pump to determine the volume of released oxygen. A turn-over number (TON) of [O_2]/[**Fe(III)-1**] = 50 was determined.

When we reduced the concentration of **Fe(III)-1** to values closer to iron levels typically found in physiological systems, however, the production of oxygen, monitored electrochemically by a Clark electrode, was insignificant relative to the amount of oxygen calculated for 100% conversion of hydrogen peroxide. Figure 1 (a) shows the yield of oxygen as a function of [**Fe(III)-1**] at [H_2O_2] = 260 mM at pH = 4.6. It can be seen that no significant oxygen production was detected at [**Fe(III)-1**] < 10 μM. An increase of the amount of catalyst resulted in an increase in oxygen formation, but in

comparison with the total yield that would be possible on the basis of the catalase stoichiometry (100% = 130 mM O_2), the formation of oxygen was less than 1%.

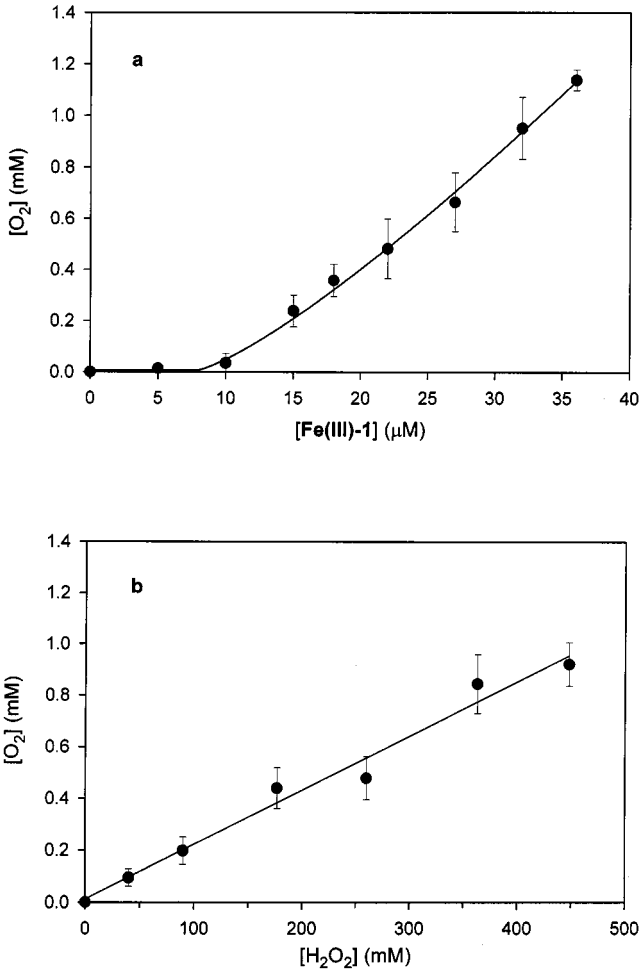

Figure 1. Oxygen yield from the reaction between **Fe(III)-1** and H_2O_2 at T = 25 °C and pH = 4.6 as: a) a function of [**Fe(III)-1**] ([H_2O_2] = 260 mM); b) a function of [H_2O_2] ([**Fe-1**] = 22 μM)

A similar conclusion follows from Figure 1 (b), in which oxygen production is determined as a function of the concentration of H_2O_2 at an **Fe(III)-1** concentration of 22 μM. From the slope of the regression line, it is possible to calculate an average relative yield of O_2 of 0.4% for this range of hydrogen peroxide concentration. Thus, although oxygen is formed by the reaction between **Fe(III)-1** and hydrogen peroxide at acidic pH,[15,16] this reaction is insignificant relative to the extent of hydrogen peroxide degradation.

Experiments similar to those with **Fe(III)-1** were carried out with complex **Fe(III)-2**, which differs from **Fe(III)-1** in the absence of the methyl groups in the macrocycle. Figure 2 displays the yield of oxygen as a function of the concentration of **Fe(III)-2** from 10 to 100 μM, for two different hydrogen peroxide concentrations (40 mM, upper line; 5 mM, lower line). At both concentrations, the amount of oxygen increases linearly with the concentration of the catalyst. At 40 mM H_2O_2 and at 100 μM **Fe(III)-2**, the yield of oxygen is only 1.2% of the total yield expected for 100% conversion of hydrogen peroxide, assuming the catalase stoichiometry. At 5 mM H_2O_2 and at the same catalyst concentration, the yield of oxygen is slightly increased, to about 5.4%. These experiments clearly demonstrate that oxygen formation, which depends both on catalyst and hydrogen peroxide concentration, is only a minor process.

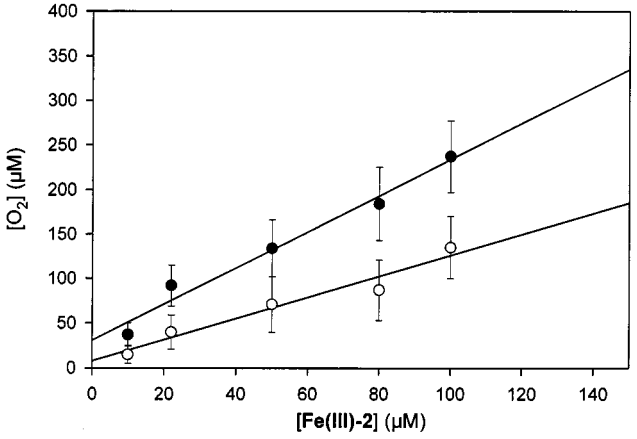

Figure 2. Oxygen yield as a function of [**Fe(III)-2**]; [H_2O_2] = 40 mM, closed circles; [H_2O_2] = 5 mM; open circles; T = 25 °C, pH = 4.6

Complex **Fe(III)-2** behaves virtually identically to **Fe(III)-1**, as is evident from Figure 3, in which oxygen formation was measured as a function of H_2O_2 concentration in the presence of 22 μM **Fe(III)-2**, comparing this with the data for the same reaction of **Fe(III)-1**. The measured yields of oxygen (average from slope: 0.42%) for the reaction between **Fe(III)-1** and hydrogen peroxide (closed circles) are virtually identical to the values (average from slope: 0.36%) found for the same reaction with **Fe(III)-2** (open circles).

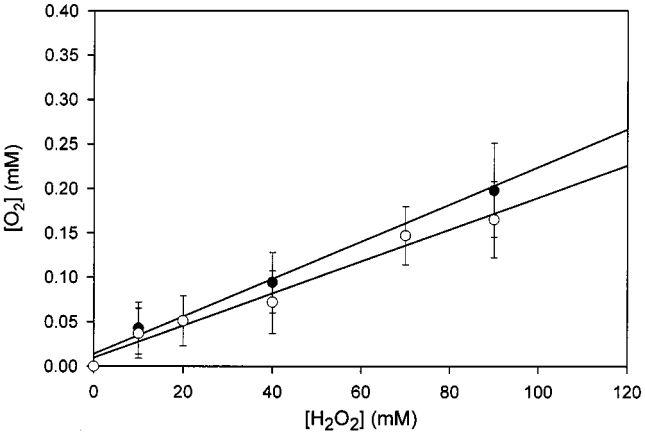

Figure 3. Comparison of the total yield of oxygen from the reactions of **Fe(III)-1** (closed circles) and **Fe(III)-2** (open circles) with H_2O_2 as a function of [H_2O_2]

More important than the formation of oxygen at pH = 4.6, however, is the question of whether oxygen is produced at a physiological pH of 7.2, a point not addressed in ref.[15,16] This property would make an enzyme mimic useful for applications in cell biology studies. Experiments addressing this question were carried out with complex

Fe(III)-2. This complex, however, does not produce detectable (≥ 0.1 μM) amounts of oxygen at pH = 7.2 and [**Fe(III)-2**] = 22 μM. Figure 4 displays the amount of hydrogen peroxide decomposed in the presence of [**Fe(III)-2**] = 22 μM as a function of hydrogen peroxide concentration. The straight line indicates theoretical 100% decomposition of hydrogen peroxide. The experiment was carried out in such a way that, 150 s after addition of **Fe(III)-2** to the buffer solution of hydrogen peroxide, the unchanged hydrogen peroxide was determined by addition of native catalase to the reaction mixture. The amount of hydrogen peroxide decomposed was then calculated from the amount of oxygen released by catalase. Control experiments revealed that after 150 s no further decomposition of hydrogen peroxide took place. It is interesting that up to 50% of hydrogen peroxide was decomposed, although no oxygen production could be detected under these conditions. Figure 4 indicates that the presence of superoxide dismutase (SOD) has no effect on the amount of H_2O_2 decomposed, and nor is any oxygen formed under these conditions. These facts largely rule out the intermediacy of superoxide radical anion in the decomposition process. Thus, **Fe(III)-2** is an active catalyst for hydrogen peroxide degradation at pH = 7.2 but cannot reasonably be considered to be a catalase mimic.

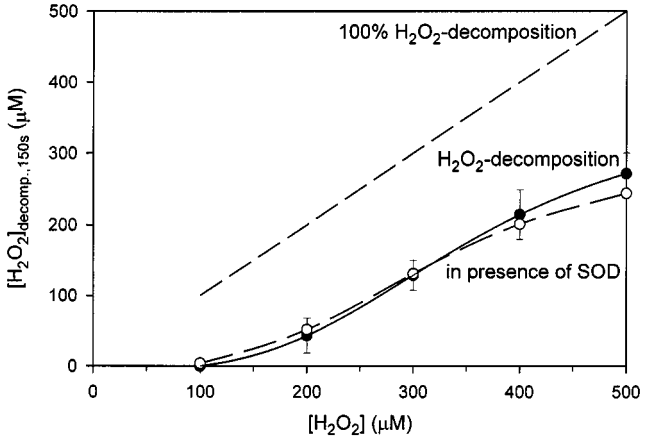

Figure 4. Decomposition of hydrogen peroxide without formation of oxygen in the presence of **Fe(III)-2**, as a function of $[H_2O_2]$ ([**Fe(III)-2**] = 22 μM; T = 25 °C; pH = 7.2), determined as the difference between the 100% value and the amount of unchanged H_2O_2 (which was measured as oxygen after addition of catalase); broken line: decomposition in the presence of SOD

In order to provide preliminary evidence for the mechanism of H_2O_2 decomposition, some exploratory experiments were performed in the presence of 2,2′-azinobis(3-ethyl-1,2-dihydrobenzthiazole-6-sulfonate) (ABTS),[19] which is frequently used to identify hydroxyl radicals in enzymatic reactions. It was concluded that free hydroxyl radicals are not released in the **Fe(III)-2**-promoted decomposition of H_2O_2. Because of the high reactivity of hydroxyl radicals, however, there exists the possibility that these species react directly with the ligand of **Fe(III)-2** in a "site-specific" manner, thus being responsible for the destruction of the catalytically active complex. Destruction of the catalytic complex by high-valent oxo−iron intermediates is also a possible scenario. Exploratory ^{1}H NMR experiments in this direction were performed. After termination of the reaction between **Fe(III)-2** and H_2O_2, several new signals that indicated an unspecific attack on the ligand were observed. The chemical shifts of the new signals point to oxygenation at the aliphatic and aromatic positions, as well as cleavage of the ring. The formation of oxygenated products would account for the loss of hydrogen peroxide without release of oxygen.

A change in reactivity towards H_2O_2 was presumed for **Fe(III)-3**, due to the presence of two aromatic residues, each directly attached to an amino group of the macrocycle. Figure 5 demonstrates that oxygen was produced in the reaction between **Fe(III)-3** and H_2O_2. At an H_2O_2 concentration of 500 μM, a linear increase of oxygen formation as a function of the concentration of **Fe(III)-3** is observed. Production of O_2 (0−11%), however, was much smaller than expected for a quantitative catalytic decomposition of H_2O_2 with respect to the catalase stoichiometry. Experiments with added catalase proved that the released O_2 fitted well with the amount of decomposed hydrogen peroxide, or in other words, no further decomposition of H_2O_2 is initiated by complex **Fe(III)-3**, unlike the cases of complexes **Fe(III)-1** and **Fe(III)-2**. Inspection of the data of Figure 5 (slope of regression line = 0.51) reveals that the reaction is stoichiometric, with one molecule of oxygen being produced per two molecules of **Fe(III)-3**.

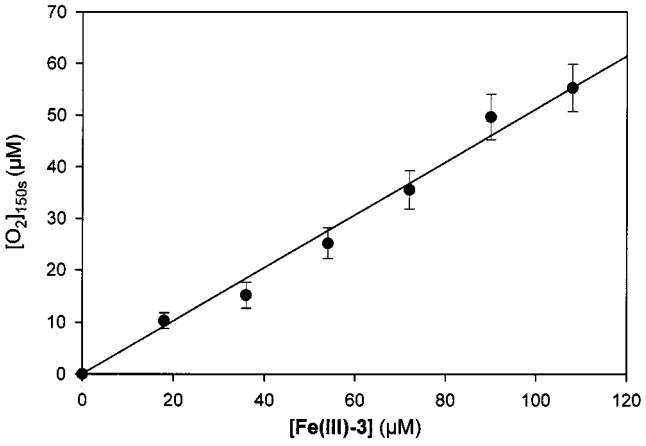

Figure 5. Oxygen yield from the reaction between H_2O_2 and **Fe(III)-3** as a function of [**Fe(III)-3**] ([H_2O_2] = 500 μM; T = 25 °C; pH = 7.2)

Conclusion

Reinvestigation of the catalase activity of tetraazamacrocyclic Fe^{III} complex **Fe(III)-1** confirmed that **Fe(III)-1** does produce oxygen at pH = 4.6 as reported previously.[15] However, it turned out that this reaction is only a minor side reaction (< 1%). Studies with the analogous complex **Fe(III)-2** showed that no oxygen is formed at a physiological pH of 7.2, despite the fact that hydrogen peroxide is decomposed by an extent of more than 50% in the presence of **Fe(III)-2** under these conditions. Complex **Fe(III)-3** does produce oxygen on interaction with hydrogen peroxide, but

only in stoichiometric amounts. Thus, while these complexes are all capable of decomposing hydrogen peroxide, the mechanisms through which H_2O_2 is destroyed seem to be different. The decomposition of hydrogen peroxide cannot reasonably be described as a "catalase-like" activity. One possible reason might be that the chemical structure of the respective ligands does not allow easy formation of a π-type radical cation, which plays a central role in the activity of natural iron-containing catalases.

Experimental Section

Instrumentation: ^1H and ^{13}C NMR spectra: Bruker GRX 500 and Varian Gemini 200. − High resolution MS: Fisons Instruments VG Pro Spec 3000 (70 eV). − Melting points (uncorrected): Elektrothermal 9100. − IR: Bio-Rad Series FTS 135 FT-IR. − UV/Vis: Varian Cary 219. − Oxygen measurements: Clark-type polarographic oxygen electrode from Eschweiler and Hansatech (oxygen electrode unit DW1 and oxygen control box CB1-D3) with DigiS data acquisition system. − Elemental analysis: Carlo Erba 1010 CHNSO.

Materials: Starting materials not mentioned below were commercially available. Catalase (beef liver, 65000 U/mg) and superoxide dismutase (bovine erythrocytes, 5000 U/mg) were purchased from Boehringer, Mannheim. The following compounds were prepared according to published procedures: {2,12-dimethyl-3,7,11,17-tetraazabicyclo[11.3.1]heptadeca-1(17),2,11,13,15-pentaene}nickel(II) perchlorate,[20] {2,12-dimethyl-3,7,11,17-tetraazabicyclo[11.3.1]-heptadeca-1(17),13,15-triene}nickel(II) perchlorate,[20] (2*R*,12*S*)-2,12-dimethyl-3,7,11,17-tetraazabicyclo[11.3.1]heptadeca-1(17),13,15-triene monohydrate (**1**),[16] pyridine-2,6-dicarbaldehyde,[21] 3,7,11,17-tetraazabicyclo[11.3.1]heptadeca-1(17),13,15-triene (**2**),[18] 1-chloro-2-nitrobenzene,[22] *N*,*N*-bis(2-aminobenzyl)amine,[23] 4-oxa-1,7-heptanediol,[24] 4-oxaheptane-1,7-ditosylate.[24]

***N*,*N*-Bis(2-nitrobenzyl)amine:** The literature synthesis[25] was improved as follows. An equimolar amount of gaseous ammonia was bubbled through an ice-cooled solution of nitrobenzyl chloride (10.0 g, 58.3 mmol) in ethanol (100 mL). The reaction mixture was allowed to stand for 6 d at room temperature. The crystalline product was filtered off and dissolved in chloroform. Insoluble ammonium chloride was filtered off and chloroform was removed by evaporation to afford the product as yellow needles (6.01 g, 26.8 mmol, 92%). − M.p. 100 °C (ref.[25] 99−100 °C). − ^1H NMR (200 MHz, $CDCl_3$): δ = 1.98 (s, 1 H, NH), 4.08 (s, 4 H, 2 × CH_2), 7.44 (m, 2 H, 2 × CH_{Ph}), 7.60 (m, 4 H, 4 × CH_{Ph}), 7.95 (m, 2 H, 2 × CH_{Ph}). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 49.8 (CH_2), 124.6 (CH_{Ph}), 124.6 (CH_{Ph}), 128.5 (CH_{Ph}), 131.7 (CH_{Ph}), 133.2 (CH_{Ph}), 134.4 (CH_{Ph}). − IR (KBr): $\tilde{\nu}$ [cm^{-1}] = 3247 (NH), 3080 (CH_{Ph}), 2909 (CH_{aliph}), 1610 (NH), 1516, 1337 (NO_2).

3,7,19,25-Tetraazatetracyclo[19.3.1.0^{4,9}0^{13,18}]pentacosa-1(25),4,6,8,13,15,17,21,23-nonaene (3): A solution of cupric nitrate trihydrate (1.62 g, 6.70 mmol) in water (20 mL) was added to a stirred solution of pyridine-2,6-dicarbaldehyde (0.91 g, 6.70 mmol) in ethanol (20 mL). A solution of *N*,*N*-bis(2-aminobenzyl)amine (1.52 g, 6.70 mmol) in ethanol (4 mL) was added dropwise to the resulting pale green solution, with rapid stirring. The reaction mixture was refluxed for 2.5 h, cooled down in an ice/brine bath, and sodium tetrahydroborate (0.66 g, 17.5 mmol) was added in small portions. After completion of addition, the solution was stirred at room temperature for 2 h, heated at 60 °C for 1 h, and finally stirred at room temperature overnight. Copper(II) was removed by treating the mixture with sodium sulfide nonahydrate (3.66 g, 15.2 mmol), followed by stirring at room temperature for 1 h, and heating at 60 °C for 1 h. The solution was cooled, and the precipitate isolated and dissolved in hot dichloromethane. Insoluble material was removed by filtration. The dichloromethane solution was cooled to 10 °C to give light yellow needles of **3** (1.79 g, 5.43 mmol, 81%). − M.p. 221 °C. − ^1H NMR (500 MHz, [D_6]DMSO): δ = 2.24 (t, ^3J = 6.3 Hz, 1 H, NH), 3.74 (d, ^3J = 6.3 Hz, 4 H, 10-H, 12-H), 4.34 (d, ^3J = 4.4 Hz, 4 H, 2-H, 20-H), 6.57 (dd, ^3J = 7.3, ^3J = 7.3 Hz, 2 H, 7-H, 15-H), 6.60 (d, ^3J = 7.9 Hz, 2 H, 5-H, 17-H), 7.11 (m, 6 H, 6-H, 8-H, 14-H, 16-H, 2 × NH), 7.37 (d, ^3J = 7.8 Hz, 2 H, 22-H, 24-H), 7.82 (dd, ^3J = 7.8 Hz, 1 H, 23-H). − ^{13}C NMR (125 MHz, [D_6]DMSO): δ = 46.4 (C-2, C-20), 54.2 (C-10, C-12), 109.9 (C-5, C-17), 115.9 (C-7, C-15), 120.2 (C-22, C-24), 124.3 (C-9, C-13), 128.6 (C-6, C-16), 130.4 (C-8, C-14), 137.5 (C-23), 147.9 (C-4, C-18), 155.5 (C-1, C21). − IR (KBr): $\tilde{\nu}$ [cm^{-1}] = 3298 (NH), 3042 (CH_{Ph}), 2843 (CH_{aliph}), 1606 (NH). − UV (dichloromethane): λ_{max} [nm] (lg ε) = 251 (4.37), 297 (3.92). − MS (70 eV): *m*/*z* (%) = 330 (66) [M^+], 209 (100) [$M^+ - C_7H_9N_2$]. − HR-MS calcd. for: $C_{21}H_{22}N_4$: 330.184447; found 330.186833.

***N*,*N*-Bis(2-cyanoethyl)-2-methoxybenzylamine:** 2-Methoxybenzylamine (21.9 g, 0.16 mol), acrylonitrile (14.5 g, 0.27 mol), and benzoquinone (0.50 g, 4.63 mmol) (to avoid formation of polymeric byproducts) were placed in a 250-mL ampoule. After flame-sealing, the reaction mixture was heated at 140 °C for 6 d. The viscous product was obtained by distillation (18.5 g, 76.0 mmol, 56%). − B.p. 185−195 °C (0.1 hPa). − ^1H NMR (200 MHz, $CDCl_3$): δ = 2.43 (t, ^3J = 6.9 Hz, 4 H, 2 × CH_2), 2.81 (t, ^3J = 6.9 Hz, 4 H, 2 × CH_2), 3.67 (s, 2 H, CH_2), 3.79 (s, 3 H, CH_3), 6.90 (m, 2 H, 2 × H_{Ph}), 7.27 (m, 2 H, 2 × H_{Ph}). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 16.7 (CH_2), 49.6 (CH_2), 51.1 (CH_2), 55.5 (CH_3), 110.7 (C_{Ph}), 119.1 (CH_{Ph}), 120.7 (CN), 125.7 (CH_{Ph}), 128.9 (CH_{Ph}), 130.6 (CH_{Ph}), 157.9 (CH_{Ph}). − $C_{14}H_{17}N_3O$ (243.3): calcd. C 69.11, H 7.04, N 17.27; found C 69.25, H 7.13, N 17.14.

***N*,*N*-Bis(3-aminopropyl)-2-methoxybenzylamine:** A solution of aluminium chloride (22.1 g, 0.17 mol) in anhydrous diethyl ether (200 mL) was added to a suspension of lithium aluminium hydride (6.34 g, 0.17 mol) in anhydrous diethyl ether (330 mL) under argon. The mixture was vigorously stirred and a solution of *N*,*N*-bis(2-cyanoethyl)-2-methoxybenzylamine (16.0 g, 65.8 mmol) in anhydrous diethyl ether (200 mL) was added over a period of 2 h. After completion of addition, the mixture was stirred for 90 h. The reaction mixture was then cooled to 0 °C and quenched with 30% potassium hydroxide (w/v). The yellow ether layer was separated and the aqueous phase was extracted with diethyl ether (5 × 50 mL). The combined ethereal extracts were dried with sodium sulfate and filtered, and the diethyl ether was evaporated. The orange, oily residue was distilled to give a viscous product (9.78 g, 38.9 mmol, 59%). − B.p. 135−142 °C (0.1 hPa). − ^1H NMR (200 MHz, $CDCl_3$): δ = 1.00 (br. s, 4 H, 2 × NH_2), 1.52 (m, 4 H, 2 × CH_2), 2.39 (t, ^3J = 6.9 Hz, 4 H, 2 × CH_2), 2.61 (t, ^3J = 6.9 Hz, 4 H, 2 × CH_2), 3.47 (s, 2 H, CH_2), 3.71 (s, 3 H, CH_3), 6.85 (m, 4 H, 4 × H_{Ph}). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 30.8 (CH_2), 40.3 (CH_2), 51.4 (CH_2), 51.86 (CH_2), 54.9 (CH_3), 109.8 (CH_{Ph}), 119.9 (CH_{Ph}), 127.3 (CH_{Ph}), 127.6 (CH_{Ph}), 129.7 (CH_{Ph}), 157.3 (CH_{Ph}). − IR (HATR): $\tilde{\nu}$ [cm^{-1}] = 3293 (NH), 3063 (CH_{Ph}), 2940 (CH_{aliph}), 2835 ($CH_{methyl\ ether}$), 1570 (NH), 1241 ($CO_{arylalkyl\ ether}$). − $C_{14}H_{25}N_3O$ (251.4): calcd. C 66.89, H 10.02, N 16.72; found C 66.74, H 10.05, N 16.53.

7-(2-Methoxybenzyl)-3,7,11,17-tetraazabicyclo[11.3.1]heptadeca-1(17),13,15-triene (4a): A solution of cupric nitrate trihydrate

(10.2 g, 42.1 mmol) in water (90.0 mL) was added to a solution of pyridine-2,6-dicarbaldehyde (5.69 g, 42.1 mmol) in ethanol (90 mL) whilst stirring. To the resulting pale green mixture was added dropwise a solution of *N*,*N*-bis(3-aminopropyl)-2-methoxybenzylamine (10.6 g, 42.1 mmol) in ethanol (18 mL), with rapid stirring. After completion of addition (30 min), the resulting dark blue solution was refluxed for 4 h, then stirred at room temperature overnight. The reaction mixture was cooled to 5 °C in an ice/brine bath and sodium borohydride (4.00 g, 0.11 mol) was added over 30 min. The solution was then stirred at room temperature for 1 h, heated at 60 °C for 1 h, and stirred further at room temperature for several hours. The copper(II) was removed by treating the mixture with sodium sulfide nonahydrate (23.1 g, 96.0 mmol). The mixture was stirred at room temperature for 1 h, and heated at 60 °C for 2 h. The solution was cooled and the copper(II) sulfide removed by filtration through Celite. Ethanol was evaporated, the residue extracted three times with dichloromethane, and the combined extracts dried with $MgSO_4$. After removal of the dichloromethane by evaporation the brown residue was purified by crystallization from chloroform (several days in a refrigerator) (8.65 g, 24.4 mmol, 58%). − M.p. 269 °C. − ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.52 (m, 4 H, 5-H, 9-H), 2.18 (t, ^3J = 6.0 Hz, 4 H, 4-H, 10-H), 2.30 (t, ^3J = 6.0 Hz, 4 H, 6-H, 8-H), 3.32 (s, 2 H, CH_2), 3.46 (s, 3 H, OCH_3), 3.67 (s, 4 H, 2-H, 12-H), 6.59 (m, 2 H, 2 × CH_{Ph}), 6.80 (d, ^3J = 7.3 Hz, 2 H, 14-H, 16-H), 6.97 (m, 2 H, 2 × CH_{Ph}), 7.34 (dd, ^3J = 7.3 Hz 1 H, 15-H). − ^{13}C NMR (125 MHz, $CDCl_3$): δ = 27.7 (C-5, C-9), 46.2 (C-4, C-10), 51.8 (*C*H_2), 52.3 (C-6, C-8), 53.5 (C-2, C-12), 55.0 (O*C*H_3), 110.1 (*C*H_{Ph}) 120.0 (*C*H_{Ph}), 120.5 (C-14, C-16), 127.3 (*C*H_{Ph}), 127.6 (*C*H_{Ph}), 130.5 (*C*H_{Ph}), 136.4 (C-15), 157.8 (*C*H_{Ph}), 159.4 (C-1, C-13). − IR (KBr): $\tilde{\nu}$ [cm^{-1}] = 3296 (NH), 3060 (CH_{Ph}), 2935 (CH_{aliph}), 2835 ($CH_{methyl\ ether}$), 1590 (NH), 1240 ($CO_{arylalkyl\ ether}$). − UV (water): λ_{max} [nm] (lg ε) = 261 (3.54), 266 (3.53), 276 sh (3.24). − HR-MS calcd. for $C_{21}H_{30}N_4O$: 354.241962 found 354.242928.

7-(2-Hydroxybenzyl)-3,7,11,17-tetraazabicyclo[11.3.1]heptadeca-1(17),13,15-triene (4b): A solution of BBr_3 in dichloromethane (1 M, 5.72 mL, 5.72 mmol) was added dropwise to an ice/brine-cooled solution of 7-(2-methoxybenzyl)-3,7,11,17-tetraazabicyclo[11.3.1]-heptadeca-1(17),13,15-triene (340.3 mg, 0.96 mmol) in anhydrous dichloromethane (8.0 mL). After completion of addition, the reaction was stirred at room temperature for 20 h, water (14.0 mL) was added dropwise in order to hydrolyze the excess of the reagent, and the mixture was stirred intensively for 2 h. The aqueous phase was extracted with dichloromethane (4 mL), brought to pH = 11 with 2.5 M NaOH, and extracted with dichloromethane (3 × 4 mL). These extracts were dried with $MgSO_4$, and the dichloromethane removed by evaporation to leave a colorless viscous product (204 mg, 0.60 mmol, 62%). − ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.74 (m, 4 H, 5-H, 9-H), 2.43 (t, ^3J = 5.8 Hz, 4 H, 4-H, 10-H), 2.48 (t, ^3J = 6.8 Hz, 4 H, 6-H, 8-H), 3.50 (s, 2 H, CH_2), 3.87 (s, 4 H, 2-H, 12-H), 6.59 (m, 1 H, CH_{Ph}), 6.66 (m, CH_{Ph}), 6.86 (m, 1 H, CH_{Ph}), 6.98 (d, ^3J = 7.8 Hz, 2 H, 14-H, 16-H), 7.01 (m, 1 H, CH_{Ph}), 7.52 (dd, ^3J = 7.8 Hz, 1 H, 15-H). − ^{13}C NMR (125 MHz, $CDCl_3$): δ = 27.1 (C-5, C-9), 45.3 (C-4, C-10), 51.5 (C-6, C-8), 53.4 (C-2, C-12), 58.4 (*C*H_2), 115.8 (*C*H_{Ph}), 118.9 (*C*H_{Ph}), 120.8 (C-14, C-16), 122.4 (*C*H_{Ph}), 128.4 (*C*H_{Ph}), 128.8 (*C*H_{Ph}), 136.8 (C-15), 156.9 (*C*H_{Ph}), 158.5 (C-1, C-13). − IR (HATR): $\tilde{\nu}$ [cm^{-1}] = 3280 (OH), (NH), 3060 (CH_{Ph}), 2932 (CH_{aliph}), 1590 (NH). − MS (70 eV): *m*/*z* (%) = 341 (75) [M^+ + 1], 247 (21) [M^+ − C_6H_5O], 233 (43) [M^+ − C_7H_7O], 107 (100) [M^+ − $C_{13}H_{21}N_4$].

Ferric Complex of 1 [Fe(III)-1]: The complex was prepared according to the method of Busch[16] (yellow powder, 49%). − IR (KBr): $\tilde{\nu}$ [cm^{-1}] = 3257, 3179 (NH), 3065 (CH_{Ph}), 2976 (CH_{aliph}), 1606 (NH), 1581 (CN_{Pyr}). − $C_{15}H_{26}Cl_2BF_4FeN_4$ (476.0): calcd. C 37.85, H 5.51, N 11.77; found C 37.56, H 5.70, N 11.51.

Ferric Complex of 2 [Fe(III)-2]: The procedure used was adapted from the literature,[16] in anhydrous methanol (total 175 mL) with an equimolar amount of ligand **2** (1.98 g, 8.46 mmol) and anhydrous ferrous chloride (1.07 g, 8.46 mmol). Tetraethylammonium tetrafluoroborate (1.84 g, 8.46 mmol) was employed as the counterion source. The ferrous complex of **2** was not isolated but directly oxidized to **Fe(III)-2** (yellow powder, 2.35 g, 5.25 mmol, 62%), decomp. at 145 °C. − ^{1}H NMR (500 MHz, D_2O): δ = 2.29 (br. s, 4 H, 5-H, 9-H), 3.20 (br. s, 4 H, 6-H, 8-H), 3.32 (br. s, 4 H, 4-H, 10-H), 4.56 (br. s, 4 H, 2-H, 12-H), 7.57 (br. s, 2 H, 14-H, 16-H), 8.01 (br. s, 1H 15-H). − ^{13}C NMR (125 MHz, D_2O): δ = 23.4 (C-5, C-9), 44.6 (C-6, C-8), 45.6 (C-4, C-10), 52.0 (C-2, C-12), 127.3 (C-14, C-16), 142.7 (C-15), 152.8 (C-1, C-13). − IR (KBr): $\tilde{\nu}$ [cm^{-1}] = 3657, 3255 (NH), 3084 (CH_{Ph}), 2945 (CH_{aliph}), 1609 (NH). − UV (water): λ_{max} [nm] (lg ε) = 254 (3.82), 276−450 br. sh. − $C_{13}H_{22}Cl_2BF_4FeN_4$ (447.9): calcd. C 34.86, H 4.95, Fe 12.47, N 12.51; found C 35.05, H 5.08, Fe 12.25 (AAS), N 12.42.

Ferric Complex of 3 [Fe(III)-3]: This compound was prepared as described above for **Fe(III)-2**. **Fe(III)-3** was obtained as a green powder (54%). − IR (KBr): $\tilde{\nu}$ [cm^{-1}] = 3630 (NH), 3059 (CH_{Ph}), 2931 (CH_{aliph}), 1633 (NH), 1060 (BF_4). − UV (dichloromethane): λ_{max} [nm] (lg ε) = 272 (4.36), 273−400 br. sh. − $C_{21}H_{22}Cl_2BF_4FeN_4$ (544.0): calcd. C 46.37, H 4.08, N 10.30; found C 46.02, H 3.89, N 10.21.

Attempted Preparation of the Ferric Complex of 4a [Fe(III)-4a]: Numerous attempts to prepare the iron(III) complex of **4a** were made, by variation of the reaction conditions as summarized here. Iron(II) salts: $FeCl_2$, $Fe(CH_3CO_2)_2$, $Fe(ClO_4)_2$·6 DMSO, and $Fe(BF_4)_2$ and subsequent oxidation by air to Fe^{III}, with BF_4^-, ClO_4^-, $B(C_6H_5)_4^-$, or PF_6^-, respectively, as counter-ion; iron(III) salts: $FeCl_3$, $Fe(ClO_4)_3$·6 H_2O, and $Fe(ClO_4)_3$·6 DMSO; solvents (dry): methanol, ethanol, tetrahydrofuran, acetonitrile; bases: triethylamine; concentration ratios of ligand/$Fe^{II/III}$ salt = 2:1, 1:1, 1:2, 1:10; reaction times: 10 min, 1 h, 2 h, 1 d, 1 week; reaction temperatures: 20 °C, 40 °C, reflux; pressure: 1 bar, 10 kbar. In no case could a well-defined **Fe(III)-4a** complex be isolated.

Oxygen Measurements: To monitor the dioxygen evolution initiated by **Fe(III)-1**, an Eschweiler M100LCD polarographic Clark-type oxygen electrode was used. A Hansatech DW1/CB1-D3 electrode was used for **Fe(III)-2** and **Fe(III)-3**. All solutions were purged with N_2 or Ar before being introduced into the chamber. A magnetic stirrer and the electrode membranes were placed at the bottom of the reaction vessel, which was surrounded by a water jacket connected to a thermostated reservoir maintained at 25.0±0.2 °C. − The Eschweiler M100 device was calibrated by flushing the buffer solution with N_2 and O_2, respectively, to read the zero and maximal concentrations of oxygen in the solution. The Hansatech DW1/CB1-D3 device was calibrated with sodium dithionite to identify the discrepancy between zero oxygen and the electrical zero. $Na_2S_2O_4$ consumes O_2 according to the equation $Na_2S_2O_4 + O_2 + H_2O \rightarrow NaHSO_4 + NaHSO_3$. The electrical current generated by the reduction of oxygen at the cathode is converted into a voltage output signal. In order to convert the voltage output reading of the instrument to concentration units (μM), a linear calibration curve was established with known oxygen concentrations. For this purpose, the reactor was filled to a volume of 1.0 mL with varying H_2O_2 concentrations (100 μM, 200 μM, 300 μM, 400 μM, and 500 μM) in phosphate buffer. After addition of pure beef catalase (1 μL

injection volume), the output signal was correlated with the known oxygen concentration. − Oxygen release initiated by complexes **Fe(III)-1**−**Fe(III)-3** was carried out similarly, by injection of an aliquot of a 1 mM stock solution of the complex in buffer [a buffer/DMSO (99:1) solution was used in the case of **Fe(III)-3**] to a measured volume of buffer solution containing the desired amount of hydrogen peroxide, to give a total volume of 1.00 mL.

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