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Catalase Modeling with Metalloporphyrin Complexes Having an Oxygen Ligand in a Proximal Position. Comparison with Complexes Containing a Proximal Nitrogen†

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Models of catalase having a proximal oxygen ligand are described. Their ability to catalyze the dismutation of hydrogen peroxide or the oxygenation of an olefin are reported and compared to similar catalysts having a proximal nitrogen ligand. Manganese complexes are always more efficient for both oxygenase and dismutase activities than the corresponding iron derivatives. Whereas molecules having a proximal nitrogen ligand catalyze both reactions, dismutation and oxygenation, complexes having a proximal oxygen ligand catalyze mainly the dismutation reaction in the case of manganese derivatives and *only* this reaction in the case of iron complexes, the "true models" of catalase. These data suggest that, among other factors, one of the possible roles of the tyrosinato ligand in catalase is to inhibit any possible oxygen transfer reaction from the compound I species, in order to avoid an oxidative degradation of the protein.

For aerobic living systems, superoxide dismutase[1] and catalase[2] are the key enzymes involved in the control of superoxide anion and hydrogen peroxide, two species derived from the reduction of molecular oxygen and precursors of the highly toxic hydroxyl radical. In many organisms the catalase activity is related to a hemoprotein[3] (see ref 3a for the X-ray structure of the beef liver catalase), but recently Mn-containing catalases have been also isolated in some microorganisms.[4] In the case of heme catalase, the proximal atom is an oxygen from a tyrosine residue. With other factors (distal effects, protein structure, etc.) this proximal ligand contributes to define the catalytic characteristics of the enzyme compared to peroxidases, like horseradish peroxidase and cytochrome *c* peroxidase, which both have a nitrogen from histidine as a proximal ligand.[5] Catalase is difficult to reduce to the ferrous state, and the high affinity of the protein for NADPH† is not to facilitate the reduction of the native enzyme but to prevent and reverse the accumulation of compound II, an inactive form of catalase that is slowly generated during the hydrogen peroxide dismutation reaction.[6]

The oxidation of catalase and peroxidase by H_2O_2 is a two-electron process leading to compound I, a high-valent iron(IV)-oxo species with a radical cation delocalized on the porphyrin ring.[7] With the same peroxide cofactor, the main catalytic activity is different for these two types of hemoproteins: catalase is highly efficient for the hydrogen peroxide dismutation and is only able to oxidize low molecular weight alcohols[8] (e.g. methanol, ethanol), whereas horseradish peroxidase has no catalase activity and is very active in one- or two-electron oxidations of various molecules.[9] Modeling studies might help in the understanding of the tuning of the catalytic activities in heme enzymes.

Synthetic metalloporphyrins have been used to model catalase,[10] but little attention has been paid for the role of the proximal oxygen atom.[11] Here we report a more detailed study on the influence of the proximal ligand on the dismutation of hydrogen peroxide catalyzed by metalloporphyrin complexes having an oxygen or a nitrogen atom as an axial ligand.

Experimental Section

Materials. GC analyses were performed with an Intersmat IGC 120 FID instrument equipped with a 6 ft × 1.125 in. column packed with 10% Carbowax 20M on Chromosorb WHP 80-100 mesh from Alltech. UV-visible spectra were recorded on a Cary 2300 spectrophotometer.

All chemicals used were of reagent grade, from Aldrich or Fluka. High-quality dichloromethane was used without any further purification. Water was distilled before use. Hydrogen peroxide (Prolabo, 30% in aqueous solution) was titrated by iodometry. (*meso*-Tetraphenylporphyrinato)manganese(III) acetate (**6**) was from Strem Chemicals.

Synthesis of Porphyrin Ligands. The ligands corresponding to complexes **7-10**, *meso*-tetramesitylporphyrin and *meso*-tetrakis(2,6-dichlorophenyl)porphyrin, were synthesized in the laboratory by using a modified Lindsey preparation.[12a,b]

Porphyrin Corresponding to Complexes 1 and 2. 5α,15α-[2,2′-(decamethylenebis(carbonylamino))diphenylene]-10β,20β-{2,2′-[(4,4′-phenoxy-3,5-diyl-bis((butylcarbonyl)amino)]diphenylene}porphyrin. This compound was prepared by following the procedure described for the synthesis of functionalized "basket-handle" porphyrins.[13c] A solution of 4,4′-(1-methoxy-3,5-phenylene)dibutyric acid (210 mg, 0.75 mmol) in toluene (5 mL) was treated with excess of oxalyl chloride (1 mL) for 15 min. The solvent was evaporated to dryness. The residue was taken up in dry tetrahydrofuran (30 mL) and added to a solution of 5α,15α-[2,2′-(dodecamethylenebis(carbonylamino))diphenylene]-10β,20β-bis(2-aminophenyl)porphyrin[13a] (521 mg, 0.6 mmol) and triethylamine (0.25 mL) in the same solvent at room temperature. The coupling reaction was monitored by thin-layer chromatography on silica gel plates. The reaction was stopped when the single face hindered porphyrin was disap-

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† Abbreviations used in this paper: NADPH = nicotinamide adenine dinucleotide phosphate; GC = gas chromatography; BDTAC = benzyldimethyltetradecylammonium chloride; for abbreviations of basket-handle porphyrins, see Figure 1; TPP, TMP, and TDCPP = *meso*-tetraphenylporphyrinato, *meso*-tetramesitylporphyrinato, and *meso*-tetrakis(2,6-dichlorophenyl)porphyrinato, respectively; Im = imidazole; "Mn/N-base" represents a catalytic system using a manganese porphyrin and a nitrogen base as axial ligand.

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peared. After usual workup, the porphyrin was isolated by column chromatography on silica gel. Elution with a mixture of dichloromethane–acetone (3/1 v/v) gave a major red band corresponding to the methoxyphenylene derivative, which was crystallized from dichloromethane–hexane (472 mg, 70.5%). Anal. Calcd for $C_{71}H_{68}N_8O_5$: C, 76.6; H, 6.16; N, 10.06. Found: C, 76.4; H, 6.36; N, 9.83. ^{1}H NMR in $CDCl_3$ (ppm): 8.8 (m, 8 × H pyr), 8.6–7.3 (m, 16 × *o*-, *m*- and *p*-H Ph), 6.67 (s, 2 × NHCO), 6.46 (s, 2 × NHCO), 5.96 (s, 2 × *o*- and *o'*-H phenoxymethyl), 3.69 (s, 1 × *p*-H methoxyphenylene), 3.63 (s, 3 × Ph–O–CH_3), 1.6 to −0.5 (32 × methylene), 2.66 (s, 2 × NH).

This compound was treated with boron tribromide–methyl sulfide complex (3 mL) (Aldrich, 2 M in CH_2Cl_2) at 90 °C in 1,2-dichloroethane (50 mL) for 10 h.[14] The reaction mixture was diluted with dichloromethane (50 mL), washed with water, and dried (Na_2SO_4). The title compound was isolated from preparative thin-layer chromatography over silica gel plates (Merck, silica gel 60, 2 mm) with a mixture of dichloromethane–acetone (5/1 v/v) (253 mg, 55%). Anal. Calcd for $C_{70}H_{66}N_8O_5 \cdot 1.5H_2O$: C, 74.6; H, 6.17; N, 9.95. Found: C, 74.7; H, 6.13; N, 9.96. ^{1}H NMR in $CDCl_3$ (ppm): 8.78 (m, 8 × H pyr), 8.6–7.2 (m, 16 × *o*-, *m*-, and *p*-H Ph), 6.7 (s, 2 × NHCO), 6.43 (s, 2 × NHCO), 5.61 (s 2 × *o*- and *o'*-H phenol), 3.78 (s, 1 × *p*-H phenol), 1.6 to −4.5 (32 × methylene), 2.6 (s, 2 × NH).

Porphyrin Corresponding to Complexes 3 and 4. 5α,15α-[2,2'-(dodecamethylenedioxy)diphenylene]-10β,20β-[2,2'-(6-hydroxyundecamethylene-1,9-dioxy)diphenylene]porphyrin was prepared and purified by following the published procedure.[13c] (See ref 13b for a general procedure of synthesis of basket-handle porphyrins with ether linkages.)

Porphyrin Corresponding to Complex 5. 5α,15α-[2,2'-(Dodecamethylenedioxy)diphenylene]-10β,20β-[2,2'-[6-(imidazol-1-yl)undecamethylene-1,9-dioxy]diphenylene]porphyrin. This ligand was prepared and purified following the published procedures.[13b,d]

Synthesis of Porphyrin Complexes. Chloromanganese(III) and Chloroiron(III) Complexes 7–10. The insertion of the metal ion was obtained in high yield by reaction of the appropriate metal(II) salt (5-fold excess) with the free-base porphyrin, in the presence of 5 equiv of 2,4,6-collidine per porphyrin molecule, in refluxing dimethylformamide. $FeCl_2$ was used for metalation with iron, and $Mn(OAc)_2 \cdot 4H_2O$ was used for metalation with manganese. In this case, the exchange of the counterion of the complex from acetate to chloride was obtained by shaking a solution of the acetate complex in dichloromethane with a 6 M solution of hydrochloric acid. The metalated porphyrins were purified by chromatography on a basic alumina column by using dichloromethane as the eluent.

Manganese(III) Complex 1. Insertion of manganese in free-base porphyrin was accomplished with $MnCl_2$ in dimethylformamide and 2,6-lutidine at 140 °C for 2 h under argon. After evaporation of the solvent to dryness the residue was dissolved in dichloromethane, and the mixture was washed with water and dried (Na_2SO_4). The product was chromatographied on a 1 × 20 cm silica gel column, eluting with a mixture of dichloromethane–acetone (1/1 v/v). The major fraction was collected, and after evaporation to dryness, it was crystallized from dichloromethane–hexane to afford the manganese porphyrin **1** (82%). UV–visible data in toluene [λ, nm (ε, L mmol^{-1} cm^{-1})]: 374.5 (47.2), 403 (36.7), 408.5 (106.5), 527.5 (5.5), 583 (9.8), 617 (7.6).

Iron(III) Complex 2. This complex was synthesized in dimethylformamide by using anhydrous $FeCl_2$, as previously reported.[13a] It was purified by chromatography on silica gel column eluting with a mixture of dichloromethane–acetone (2/1 v/v) and crystallized from dichloromethane–hexane (81%). UV-visible data in toluene [λ, nm (ε, L mmol^{-1} cm^{-1})]: 420 (93.5), 509 (12.1), 580 (4.1), 651 (3.3).

Manganese(III) complex 3 and iron(III) complex 4 were prepared as previously described.[15]

Manganese(III) Complex 5. Insertion of manganese into the free-base porphyrin and purification of the obtained complex have been described in the literature.[13d] (See ref 13b for a general procedure of metalation of basket-handle porphyrins with ether linkages with iron.)

Experimental Conditions for the Use of Hydrogen Peroxide in Standard Dismutation Reactions. All reactions were carried out at room temperature in a 5-mL reactor equipped with a micro stirring bar. Since catalytic dismutation of H_2O_2 and epoxidation of cyclohexene were not significantly affected by molecular oxygen, they have been performed under an air atmosphere. CH_2Cl_2 (1 mL) was added to the metalloporphyrin (1.56 μmol), phase-transfer catalyst benzyldimethyltetradecylammonium chloride (BDTAC, 6.25 μmol), and imidazole (39 μmol) ([imidazole]/[catalyst] = 25); 2,6-di-*tert*-butylpyridine (24 μmol) was added in the case of catalysts **2** and **4** to ensure the coordination of the axial ligand on the central metal (see, below, the paragraph on the description of metalloporphyrin classes i and ii (compounds **1–4**). H_2O_2 (1070 μmol, i.e. 125 μL) of the commercial 8.55 M aqueous solution was diluted with 375 μL of distilled water and then added by syringe to the organic phase. Oxygen evolution was measured by volumetry; the reactor was linked to a graduated buret filled with water. The reaction time is 5 min. The results are reported in Table I.

Table I. Catalase Activity for Different Manganese or Iron Porphyrins

catalyst		oxygen yield in % (turnover no.)[a] M = Mn		M = Fe	
M(−Ph-O-prox)(−C_{10}−)	**1**	26[b] (89)[c]	**2**	21 (71)	
M(−Alk-O-prox)(−C_{12}−)	**3**	28 (94)	**4**	18 (64)	
M(TMP)Cl	**7**	40 (140)	**8**	10 (33)	
M(TDCPP)Cl	**9**	39 (130)	**10**	7 (23)	
M(−Im-prox)(−C_{12}−)Cl	**5**	39 (130)			
M(TPP)OAc	**6**	16 (54)			

[a] All results are obtained after 5 min of reaction. Relative errors are ranging from 5 to 9%. [b] Molecular oxygen yield is based on the initial amount of hydrogen peroxide. [c] Turnover numbers are based on the molecular oxygen yield and correspond to the number of produced oxygen molecules per catalyst molecule. For details on experimental conditions, see the Experimental Section.

Experimental Conditions for Standard Competitive Reactions. The conditions were identical with those used for studies of catalase activity (see above) except for the presence of 0.25 mmol of cyclohexene and 0.13 mmol of chlorobenzene as an internal standard for GC analyses ([H_2O_2]/[olefin] = 4). The reaction time is 5 min. The results are reported in Table II.

Variation of Standard Conditions for Mn(TMP)Cl-Catalyzed Experiments. With this catalyst, we have studied the influence of the imidazole/catalyst molar ratio and of the oxidant/olefin ratio. Then, starting from the standard conditions described above, we have studied the catalase activity and both catalase and oxygenase competitive activities for greater amounts of imidazole, keeping constant the concentration of catalyst (156 μmol of imidazole, i.e. 100 molecules/catalyst molecule), and greater amounts of cyclohexene, keeping constant the oxidant concentration (1060 μmol of cyclohexene, i.e. 1 molecule/oxidant molecule). The results are given in Table III.

Results

Description of Metalloporphyrins Used in the Studies. Biomimetic oxygenation reactions using a metalloporphyrin as catalyst are generally much more efficient when a nitrogen-base ligand, such as a pyridine or imidazole derivative, is added in the medium as cocatalyst.[16] These compounds are known to act as axial ligands trans to the metal-oxo active species and then reproduce one part of the environment of the active site of the enzyme and help to transfer the active oxygen to the substrate.

In order to mimic in a more precise way the catalytic site of the catalase, we have used oxygen and nitrogen ligands covalently linked to the metal ion of the metalloporphyrin. These ligands belong to a chain attached to two trans phenyl groups of the tetraarylporphyrin via ether or amide linkages. The other side of the metalloporphyrin plane was hindered by a hydrocarbon chain.

Compounds **1–10**, used as catalysts, can be classified in four categories (Figure 1): (i) manganese and iron complexes with an oxygen atom from a proximal phenoxo ligand and a distal chain attached to the phenyl groups via amide linkages (compounds **1** and **2**), (ii) manganese and iron complexes with an oxygen atom from a proximal alkoxo ligand and a distal hydrocarbon chain attached to the phenyl groups via ether linkages (compounds **3** and **4**), (iii) a manganese complex, **5**, with a nitrogen from a proximal imidazole ligand and the same distal chain as in derivatives **3** and **4**, and (iv) various metallotetraarylporphyrins with no prepositioned nitrogen or oxygen ligands in axial positions. Among these last compounds, those with a tetramesitylporphy-

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Table II. Competitive Oxygenation and Dismutation Reactions

catalyst		M = Mn		M = Fe	
no.	compd	catalase[c] oxygen yield, %	oxygenase[c] epoxide yield, %	catalase[c] oxygen yield, %	oxygenase[c] epoxide yield, %
1,[a] **2**[b]	M(−Ph-O-prox)(−C_{10}−)	26[d] (89)[e]	0	18 (60)	0
3[a], **4**[b]	M(−Alk-O-prox)(−C_{12}−)	27 (93)	1[f] (2)[g]	21 (70)	0
5[a]	M(−Im-prox)(−C_{12}−)Cl	31 (110)	4 (6)		
7,[a] **8**[b]	M(TMP)Cl	27 (93)	33 (53)	12 (40)	0
9,[a] **10**[b]	M(TDCPP)Cl	28 (100)	20 (32)	9 (30)	0

[a] Manganese complexes. [b] Iron complexes. [c] All results are obtained after 5 min of reaction. Relative errors range from 5 to 9%. [d] Molecular oxygen yield is based on the initial amount of hydrogen peroxide. [e] Turnover numbers (in parentheses) are based on the molecular oxygen yield and correspond to the number of produced oxygen molecules per catalyst molecule. [f] Epoxide yield is based on the initial amount of olefin. [g] Turnover numbers (in parentheses) are based on the epoxide yield and correspond to the number of produced epoxide molecules per catalyst molecule. For details on experimental conditions, see the Experimental Section.

Table III. Competition between Oxygenase and Catalase Activities of the Mn(TMP)Cl/H_2O_2/Imidazole System

				epoxide[a]		O_2[a]	
run	[Im]/[catalyst]	[H_2O_2]/[olefin]	R[b]	yield, %	μmol	yield, %	μmol
1	25	no olefin[g]				40[e] (140)[f]	210
2	100	no olefin				45 (160)	240
			Olefin Deficient vs Oxidant				
3	25	4[g]	1.8	33[c] (53)[d]	83	27 (93)	140
4	100	4	2.5	26 (45)	70	33 (110)	170
			Olefin in Equal Quantity vs Oxidant				
5	25	1	1.6	10 (65)	100	30 (100)	160
6	100	1	3.2	6 (42)	66	39 (130)	210

[a] All results are obtained after 5 min of reaction. Relative errors range from 5 to 9%. [b] R stands for the ratio of molecular oxygen/epoxide turnover numbers. [c] Molecular oxygen yield is based on the initial amount of hydrogen peroxide. [d] Turnover numbers (in parentheses) are based on the molecular oxygen yield and correspond to the number of produced oxygen molecules per catalyst molecule. [e] Epoxide yield is based on the initial amount of olefin. [f] Turnover numbers (in parentheses) are based on the epoxide yield and correspond to the number of produced epoxide molecules per catalyst molecule. For details on experimental conditions, see the Experimental Section. [g] These are standard experimental conditions.

rinato or a tetrakis(2,6-dichlorophenyl)porphyrinato ligand (TMP or TDCPP) have been successfully used in P-450 modeling, since these sterically hindered porphyrins create a sort of open well around the metal–oxo active species. Moreover, their complexes are stable under a large range of oxidative conditions.[17-19]

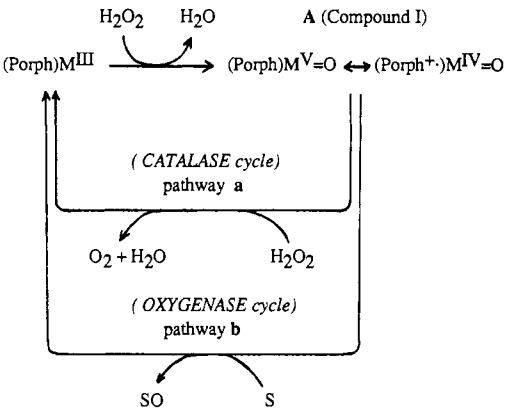
For the two first classes of complexes, with an oxygen atom as a proximal ligand, it has previously been shown that the coordination of the oxygen atom on the metal ion is dependent on two factors: the central atom itself and the basicity of the metalloporphyrin solution. In fact, two forms of the complexes are in equilibrium: one "free OH", with a free alcohol or phenol function, and one "O-linked", with the oxygen atom linked to the metal. In the case of manganese (complexes **1** and **3**), the equilibrium is completely shifted to the O-linked form, whereas 15 equiv of a noncoordinating base (2,6-di-*tert*-butylpyridine) were added to ensure the total coordination of the axial ligand on the iron (complexes **2** and **4**).[11,13e] Moreover, we checked by UV–visible spectroscopy that the addition of imidazole does not displace the internal alkoxo or phenoxo proximal ligand in complexes **1**–**4**.

The activity of all these described catalysts, especially the role of the different proximal ligands, will be discussed in terms of oxygenase versus dismutase activities (see Scheme I). In fact, an oxygen atom transfer should be called a peroxygenase reaction; however we will use the term oxygenase throughout the present article.

The catalase activity of the system was then evaluated by the measurement of the evolution of molecular oxygen (it had been previously checked with an oxygraph that the gas emission was really molecular oxygen). The oxygenase activity could be easily measured by the conversion into epoxide of an olefin (cyclohexene) present in the medium.

The experimental conditions (different compounds ratios, reaction times, etc.) have been adjusted for the system by using (tetramesitylporphyrinato)manganese(III) chloride, Mn(TMP)Cl,

Scheme I. Catalase Cycle versus Oxygenase Cycle for Metal–Oxo Porphyrins Generated by Hydrogen Peroxide



as catalyst (see below, section 3). But it is important to point out that, even with a "coordinated axial ligand catalyst", the presence of free imidazole in the medium is necessary. In fact, this base is not only a possible axial ligand for the central metal ion, but it can play the role of a distal ligand, allowing the heterolytic cleavage of the O–O bond of the hydrogen peroxide, thus leading to the high-valent metal–oxo "compound I". This assistance can be compared to the "push–pull mechanism" of catalase itself (for a reference on the push–pull mechanism for peroxidases, see ref 5a). Then, in all presented experiments, 25 equiv of imidazole/catalyst molecule have been used as cofactor. At such ratio, nearly all the TMP- or TDCPP–manganese complexes exist as the monoimidazole adducts (see ref 16d and cited references for a discussion on this particular point).

1. Catalase Activity of Different Manganese or Iron Porphyrins. In the biphasic medium described in the Experimental Section, different porphyrins with a proximal oxygen atom from an alkoxo or a phenoxo ligand or a nitrogen from imidazole have been used as catalysts for the dismutation of H_2O_2. Since all metalloporphyrins used are only soluble in dichloromethane, all catalytic

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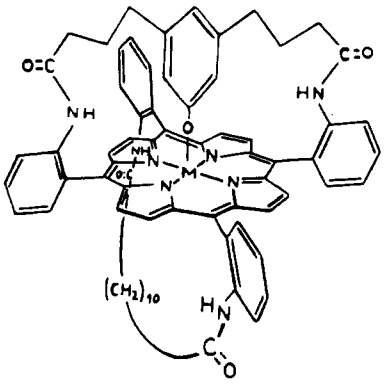
(i) : M(-Ph-O-prox)(-C10-)

1. M = Mn

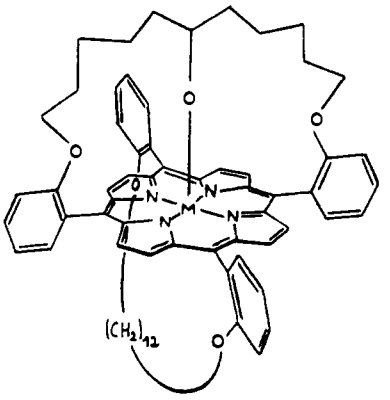
2. M = Fe



(ii) : M(-Alk-O-prox)(-C12-)

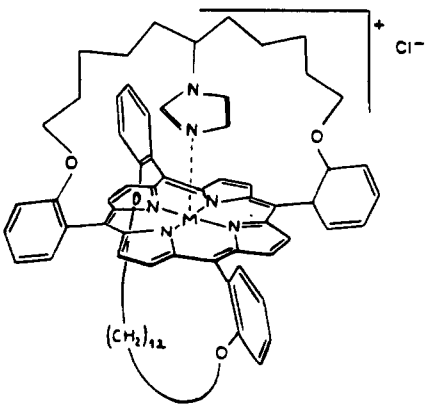
3. M = Mn

4. M = Fe



(iii) : M(-Im-prox)(-C12-)Cl

5. M = Mn



(iv) : Various metallotetraarylporphyrins

6. M = Mn; $R_1 = R_2 =$ -H; X = -OAc Mn(TPP)OAc

7. M = Mn; $R_1 = R_2 = -CH_3$; X = -Cl Mn(TMP)Cl

8. M = Fe; $R_1 = R_2 = -CH_3$; X = -Cl Fe(TMP)Cl

9. M = Mn; R_1 = -Cl; R_2 = -H; X = -Cl Mn(TDCPP)Cl

10. M = Fe; R_1 = -Cl; R_2 = -H; X = -Cl Fe(TDCPP)Cl

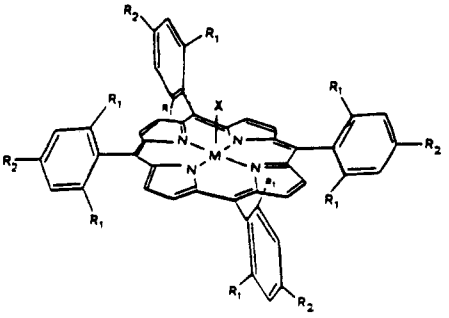


Figure 1. Different metalloporphyrins used as catalysts.

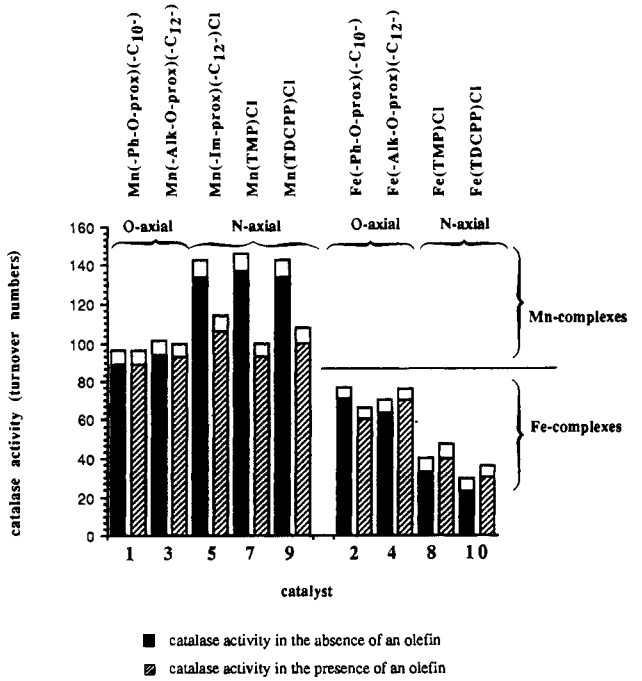
reaction described occurred in the organic phase. High yields of O_2 are obtained, assuming that two molecules of hydrogen peroxide are required for the formation of one molecule of oxygen. The results in Table I compare the activity of manganese with respect to the analogue iron complexes on one hand and that of different porphyrin ligands on the other hand.

Manganese porphyrin complexes are generally more active than the iron ones for the dismutation of hydrogen peroxide, including the Fe(-Ph-O-prox)(-C_{10}-) complex, which can be considered as a "true model" for catalase (an oxygen atom from tyrosine linked to Fe^{3+}). All presented data correspond to a reaction time of 5 min. Within this period, the oxidative degradation of the different complexes is minimum. The catalytic activities of the manganese derivatives, **1**, **3**, **7**, and **9**, are in the range of 89–140 cycles in 5 min, whereas the iron complexes, **2**, **4**, **8**, and **10**, are less active (23–71 cycles for the same period of time). In addition, it must be noted that complex **2**, the true model of catalase, has nearly the same dismutase activity as the iron analogue complex **4** with an alkoxo instead of a phenoxo ligand (71 and 64 cycles in 5 min for **2** and **4**, respectively).

2. Competition between Catalase and Oxygenase Activities with Different Metalloporphyrins. In the presence of an olefin (cyclohexene) we measured, for a family of metalloporphyrins, both yields of molecular oxygen and epoxide due to the two competitive reactions. From the results, summarized in Table II, it can be seen that, even in the presence of an olefin, all manganese porphyrins are more efficient for the hydrogen peroxide dismutation than their iron analogues. This is the case of the manganese complex with an axial phenoxo ligand, **1**, compared to its iron analogue **2**: 89 catalase cycles within 5 min for **1** instead of 60 cycles for **2**. The same trend is observed for manganese complexes **3** (with a proximal alkoxo ligand), **7**, and **9** compared to the corresponding iron complexes **4**, **8**, and **10**: 93 catalase cycles for **3** compared to 70 for **4**, 93 cycles for **7** compared to 40 cycles for **8**, and 100 cycles for **9** compared to 30 for **10**. "Basket-handle" complexes **1**–**4**, having a limited access to the metal center due to the aliphatic chain, are still able to catalyze the dismutation of hydrogen peroxide, but their oxygenase activity is more than poor. It is only 2 cycles for complex **3**, and no oxygenase activity is observed for complexes **1** and **2** (these two complexes have a phenoxo proximal ligand and are more resemblant to the catalase active site).

3. Catalase and Oxygenase Activities during the H_2O_2 Epoxidation of Cyclohexene Catalyzed by Mn(TMP)Cl (7) in the Presence of Imidazole. The results of competitive hydrogen peroxide dismutation and cyclohexene epoxidation are reported in Table III for different ratios of imidazole/catalyst and of oxidant/olefin. The increase of imidazole quantity from 25 to 100 molecules per catalyst produced an increase of oxygen evolution and a slight diminution of the epoxide yield (run 3 compared

Chart I. Catalase Activity and Catalase versus Oxygenase Activity of Different Metalloporphyrin Catalytic Systems[a]



[a] The white rectangle at the top of each bar indicates the estimated error value.

to run 4 and run 5 compared to run 6). This diminution can be due to the competition between the oxygenation of olefin and the oxidative degradation of imidazole in the medium (such imidazole oxidation by high-valent metal–oxo species was previously mentioned when NaOCl[16] and ROOH[20] were used as primary oxidants).

The most interesting result is that an increase of the amount of olefin (i.e. when the H_2O_2/olefin ratio is 1/1 instead of 4/1, keeping constant the oxidant concentration, has a small effect on the molecular oxygen yield (it increases from 27% to 30% for 25 molecules of imidazole per catalyst molecule, run 3 compared to run 5, and from 33% to 39% for 100 molecules of imidazole per catalyst molecule, run 4 compared to run 6). Similarly the epoxide yield decreases from 33% to 10%, run 3 compared to run 5, and from 26% to 6%, run 4 compared to run 6. When hydrogen peroxide is the limiting factor (runs 5 and 6), the oxygenase activity is always much more affected, in fact reduced, than the dismutase activity. This clearly indicates that the dismutation reaction rate is higher than the cyclohexene epoxidation rate. Because of this difference in both reaction rates, it is more reasonable to compare the oxygenase and the dismutase activities at the early stage of the reaction. For this reason we chose to determine the turnover numbers of both reactions within the first 5 min (in addition, oxidative degradation of porphyrin complexes might obscure the results for longer reaction times).

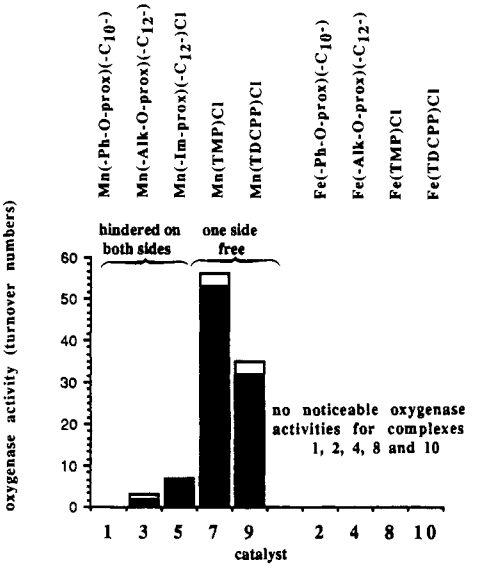
With 25 molecules of imidazole per catalyst molecule, the catalase activity/oxygenase activity ratio is 93/53 cycles, i.e. 1.8, when the olefin is in default with respect to the oxidant (run 3). This ratio is 100/65, i.e. 1.6, when the olefin is in equal quantity with respect to the hydrogen peroxide (run 5). With 100 molecules of imidazole per catalyst, the ratios are 2.5 (run 4) and 3.2 (run 6), respectively. This is another way to illustrate the fact that the catalase activity is always higher than the oxygenase activity.

Discussion

Catalase Activity of Different Manganese or Iron Porphyrins. It is clear that, among the manganese complexes, the ones having a nitrogen atom as axial ligand, Mn(TMP)Cl, Mn(TDCPP)Cl, or Mn(-Im-prox)(-C_{12}-)Cl, are more active than those with an

(20) Balasubramanian, P. N.; Sinha, A.; Bruice, T. C. *J. Am. Chem. Soc.* **1987**, *109*, 1456–1462.

Chart II. Oxygenase Activity of Different Metalloporphyrin Catalytic Systems[a]



[a] The white rectangle at the top of each bar indicates the estimated error value.

oxygen—either from alkoxo or phenoxo—as axial ligand, except with Mn(TPP)OAc. The rather low activity of the Mn(TPP)OAc/imidazole system is due to the rapid bleaching of the catalyst by H_2O_2. These results are presented in Chart I.

Then we can propose a classification of the catalase activity depending on the nature of the coordinated proximal ligand:

Mn(-Im-prox) > Mn(-Alk-O-prox) ≈ Mn(-Ph-O-prox) > Fe(-Ph-O-prox) ≈ Fe(-Alk-O-prox)

Competition between Catalase and Oxygenase Activities with Different Metalloporphyrins. From the results listed in Table II, it can be noted that even when manganese complexes are catalyzing *both* oxygenase and dismutase reactions, they are more effective for hydrogen peroxide dismutation than the corresponding iron complexes. This is true not only for manganese complexes with a proximal oxygen atom but also for those having a nitrogen axial ligand. The second feature is that manganese complexes with a nonprecoordinated axial ligand, **7** and **9**, have both important catalase and oxygenase activities, whereas the basket-handle manganese porphyrins, with two sterically hindered faces, keep a good catalase activity and have a very poor oxygenase activity. But the most important fact is that iron porphyrins *have no oxygenase activity at all* (these results are illustrated in Chart II).

On the basis of these data, we can propose a classification of the oxygenase activity depending on the nature of the metal ion and of the proximal ligand:

Mn(-Im-prox) > Mn(-Alk-O-prox) > Mn(-Ph-O-prox) ≈ Fe(-Ph-O-prox) ≈ Fe(-Alk-O-prox)

It can be noticed that, among the manganese complexes, compound **1**, the one with a phenoxo proximal ligand like in catalase itself, is the only manganese complex without any oxygenase activity.

Then it is clear that iron porphyrins with an oxygen as coordinated proximal ligand—even if they do not have the best catalase activity—are the systems that, reducing until zero the epoxide yield, have the best selectivity between the two possible reactions: dismutation and oxygenation. Oxygenase activities have been reported by Mansuy et al.[21a] for manganese and iron porphyrins

(21) (a) Renaud, J. P.; Bartoli, J. F.; Reina-Artiles, M.; Fort, M.; Mansuy, D. *J. Am. Chem. Soc.* **1988**, *110*, 8462–8470. (b) Anelli, P. L.; Banfi, S.; Montanari, F.; Quici, S. *J. Chem. Soc., Chem. Commun.* **1989**, 779–780. (c) Banfi, S.; Maiocchi, A.; Montanari, F.; Quici, S. *Gazz. Chim. Ital.* **1990**, *120*, 123–130.

using hydrogen peroxide as oxygen source. However, these catalytic reactions were performed in a mixture of acetonitrile and dichloromethane over a reaction time of 1 h. During the course of the present work, efficient metalloporphyrin-catalyzed H_2O_2 olefin epoxidations were reported by Montanari et al.[21b,c] for reactions run in the presence of a small amount of benzoic acid; in this case, the highest catalytic activities were observed with Mn(TDCPP)Cl as catalyst.

The absence of oxygenase activity cannot be attributed, in the present case, to the formation of a bis(imidazole) adduct with iron-porphyrin complexes, since the dismutation reaction is still catalyzed by the iron complexes in the same reaction conditions. In addition, it must be noted that the oxygenase activity is always less important with the superstructured basket-handle porphyrin complexes than with TMP or TDCPP ligands (Table II). So, a distal chain acts as a screen and enhances the steric discrimination between possible substrates for the high-valent metal-oxo intermediate complex (the approach of hydrogen peroxide, a smaller molecule than cyclohexene, to the metal-oxo species is less restricted with complexes **1-5** compared to complexes **7-10**).

Conclusion

From these studies on the catalase activity of various manganese and iron complexes, with or without a nonexchangeable axial oxygen or nitrogen ligand, the main following features can be noted: (i) the presence of free imidazole is always necessary to induce an heterolytic cleavage of H_2O_2—distal effect—and obtain efficient reactions, (ii) during competitive hydrogen peroxide dismutation and cyclohexene epoxidation reactions, the dismutation reaction is more rapid than the olefin oxygenation, (iii) all manganese porphyrins are more efficient than their iron analogues, (iv) a nitrogen base as proximal ligand favors the hydrogen peroxide dismutation compared to an oxygen atom from an alkoxo or phenoxo ligand, and (v) under our conditions, iron porphyrins have no detectable oxygenase activity.

The highest catalase activity is obtained with a synthetic system having manganese as metal and nitrogen as base, whereas the enzyme itself has iron as metal and a phenoxo from a tyrosine as axial ligand. But it is clear that the system "Mn/N-base" gives also the highest oxygenase activity, which might be destructive for the oxidizable amino acid residues of the distal side of the active site in enzyme. The iron porphyrins with an oxygen proximal ligand—especially Fe(-Ph-O-prox)(-C_{10}-), which is a true model of the catalase active site—still conserve a reasonable catalase activity and have no potentially destructive oxygenase activity at all; this could be the reason that the prosthetic group of catalase consists of an oxygen-containing ligand as a proximal ligand of a ferriprotoporphyrin IX.

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Water-Soluble Ferric Porphyrinates: Solution and Solid-State Species

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The nature of the aqueous solution axial ligands of the water-soluble iron(III) porphyrinates $[Fe(TMPyP)]^{5+}$ and $[Fe(TPPS)]^{3-}$ has been examined as a function of pH by ^1H NMR spectroscopy. The NMR shifts provide indirect evidence for the existence of $[Fe(TPPS)(H_2O)_2]^{3-}$, $[Fe(TPPS)(H_2O)(OH)]^{4-}$, and $[(Fe(TPPS))_2O]^{8-}$ in the $[Fe(TPPS)]^{3-}$ system and $[Fe(TMPyP)(H_2O)_2]^{5+}$, $[Fe(TMPyP)(H_2O)(OH)]^{4+}$, $[(Fe(TMPyP))_2O]^{8+}$, and $[Fe(TMPyP)(OH)_2]^{3+}$ in the $[Fe(TMPyP)]^{5+}$ system. Estimates of the distribution of the species from the NMR spectra allows approximation of the various equilibrium constants in the $[Fe(TPPS)]^{3-}$ system. The pH-dependent equilibria for $[Fe(TPPS)]^{3-}$ and $[Fe(TMPyP)]^{5+}$ correspond qualitatively with previously published results. Attempts to prepare crystalline species at various pH's were successful only for the high pH form of $[Fe(TMPyP)]^{5+}$. A single-crystal structure determination shows that this species has the idealized composition $[(Fe(TMPyP))_2O](ClO_4)_8 \cdot 4H_2O$. The five-coordinate iron atom has an axial Fe-O bond distance of 1.750 (2) Å, while the average $Fe-N_p$ bond distance is 2.080 (8) Å; the iron(III) atom is 0.47 Å out of the porphyrin plane. Crystal data: a = 32.06 (2) Å, b = 18.492 (9) Å, c = 17.17 (2) Å, β = 108.6 (1)°, monoclinic, space group $C2/c$, V = 9650 (2) Å^3, Z = 4, $Fe_2Cl_8O_{37}N_{16}C_{88}H_{80}$, 5188 observed data, $R_1(F_o)$ = 0.125, $R_2(F_o)$ = 0.140, all observations at 118 K.

Water molecules and hydroxide ions are potentially important ligands for iron in hemoproteins, and protein crystal structures have shown such coordinated ligands in the ligand binding pockets of a number of heme proteins.[1-5] It would thus seem desirable to define the aqueous interaction of the iron porphyrinates over a significant pH range. The aqueous solution chemistry of naturally occurring iron porphyrinates is difficult to investigate owing to their low solubility and strong tendency to aggregate in polar solvents.[6] Synthetic ferric water-soluble porphyrins have been used to avoid the aggregation and solubility problems. Aggregation is largely avoided by use of bulky peripheral groups while water solubility is obtained through charged peripheral substituents. The two most common such synthetic derivatives are the negatively charged $[Fe(TPPS)]^{3-}$ and the positively charged $[Fe(TMPyP)]^{5+}$ species.[7]

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(2) Cytochrome *c* peroxidase: Finzel, B. C.; Poulos, T. L.; Kraut, J. *J. Biol. Chem.* **1984**, *259*, 13027. Poulos, T. L.; Freer, S. T.; Alden, R. A.; Edwards, S. L.; Skogland, U.; Takio, K.; Eriksson, B.; Xuong, N.; Yonetani, T.; Kraut, J. *J. Biol. Chem.* **1980**, *255*, 575.
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(6) White, W. I. In *The Porphyrins*; Dolphin, D., Ed.; Academic: New York, 1978; Vol. 5, pp 303-335.
(7) Abbreviations used: H_2TPPS, tetrakis(4-sulfonatophenyl)porphyrin; H_2TMPyP, tetrakis(1-methylpyridinium-4-yl)porphyrin; H_2TAPP, tetrakis(*N*,*N*,*N*-trimethylanilinium-4-yl)porphyrin; H_2TPP, tetraphenylporphyrin; H_4FF, face-to-face porphyrin; H_2ODM, 5,15-dimethyl-2,3,7,8,12,13,17,18-octaethylporphyrin; NCH_3TPP, monoanion of *N*-methyl-*meso*-tetraphenylporphyrin; $H_2ProtoMe_2$, protoporphyrin IX dimethyl ester; H_2Porph, water-soluble porphyrin ligand; DMSO-d_6, dimethyl-d_6 sulfoxide; DSS, 2,2-dimethyl-2-silapentane-5-sulfonate; HIm, imidazole.

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