measure of the FDA's distribution. At Ca and at each C nucleus, z components are positive; the y component at the C in Figure 1 is negative. Multiplication of these values by nuclear charges produces forces in which the ligand nuclei will be drawn upward, but the Ca will be drawn more. Electron interaction more fully screens attractions to the anionic ligand's nuclei than the attraction to the cationic metal atom's nucleus.

Ca d and p functions with π pseudosymmetry constructively interfere away from the ligand (Figure 2) in $\tilde{A}^2E_1$'s FDA. $\langle z \rangle$ is slightly less than in the ground state's FDA. Nuclear attraction energies and forces on the nuclei are weaker, especially for Ca.

$\tilde{B}^2A_1$'s FDA consists chiefly of diffuse d functions on Ca and has small contributions from diffuse Ca p functions. Note the nodal surface (Figure 3) that extends from the Ca nucleus and cuts through the C nucleus. An in-phase relationship obtains between the lower lobe on Ca and functions describing C-H bonding. The smaller $\langle z \rangle$ indicates less polarization away from the ligand. Attraction energies to Ca and C are more negative than in the ground state's FDA. Especially significant is the negative sign of the electric field at the Ca nucleus. All of the forces on the ligand nuclei exceed those of the ground state. This FDA is less antibonding between Ca and C_5H_5 than the other two FDAs.

Ca d functions with δ pseudosymmetry practically alone contribute to the $\tilde{C}^2E_2$ FDA. $\langle z \rangle$ approximates the position of the Ca nucleus. Translation of charge to smaller z accompanies more negative nuclear attraction potentials. Coulombic interactions with the electrons and nuclei of the ligand are larger, but almost no force is exerted on Ca in the z direction for this nonbonding FDA.

The most diffuse Ca s functions are the largest contributors to the FDA of $\tilde{D}^2A_1$. This FDA is primarily a diffuse sp hybrid that has been orthogonalized to functions describing the ligand.

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High Oxygen-Evolving Activity of Rigidly Linked Manganese(III) Porphyrin Dimers. A Functional Model of Manganese Catalase

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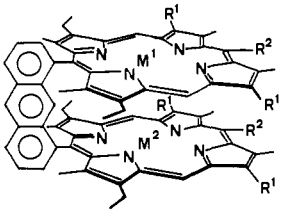
Polynuclear manganese complexes[1] play key roles in photosynthetic oxygen evolution[2] and manganese catalase (Mn-CAT).[3] Their functional relevance[4] to oxygen evolution is important both for the structural clarification of oxygen evolving complexes and for the design of their artificial models. One subunit of Mn-CAT from *Thermus thermophilus* contains two Mn^{3+} as an enzymatic active center separated by 3.6 Å.[3d] The enzyme is believed to decompose H_2O_2 by a mechanism that is unimolecular in peroxide and involves Mn^{2+} and Mn^{3+}.[3d,e] Functional modeling of this catalase activity, however, has been simulated only with a single example of a covalently linked binuclear Mn complex.[5]

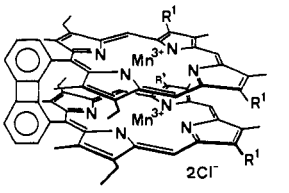
We have synthesized functional models by linking two manganese porphyrins[6] (**1**, **2b**, and **3**) by rigid linker molecules to control the metal-metal distance and their stereochemistry.



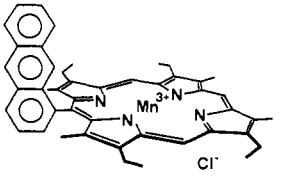
1. $R^1 = n\text{-}C_6H_{13}$, $R^2 = H$, $M^1 = M^2 = Mn^{3+}Cl^-$

2a. $R^1 = n\text{-}C_6H_{13}$, $R^2 = p\text{-}C_6H_4$, $M^1 = H_2$, $M^2 = Mn^{3+}Cl^-$

2b. $R^1 = n\text{-}C_6H_{13}$, $R^2 = p\text{-}C_6H_4$, $M^1 = M^2 = Mn^{3+}Cl^-$



3. $R^1 = n\text{-}C_6H_{13}$



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These compounds survive more than 10000 turnovers of H_2O_2 in the best case and have a cavity surrounded by porphyrin rings. The catalase activity was measured from three dimanganese complexes (**1**, **2b**, and **3**) and for their corresponding Mn porphyrin monomers [**2a**, Mn(TPP)Cl, and **4**] in a thermostated reaction cell fitted with an oxygen electrode (Table I). In the absence of 1-methylimidazole (MeIm), every Mn porphyrin showed almost no catalase activity. Remarkably, the Mn porphyrin dimers **1** and **2b** showed high oxygen-evolving activity with increased imidazole concentration. **2b** especially attained very high activity [325 mol of O_2 (mol of catalyst)$^{-1}$ min^{-1}] and high turnover numbers (maximum 1.5×10^4). For other Mn porphyrins, MeIm had little or no effect on their catalase activity. By means of spectrophotometric titration, each Mn porphyrin monomer in **1** and **3** forms only the corresponding five-coordinate complex with MeIm.[7] Complexes **1**, **2b**, and **3** have cavities made up of two bulky porphyrins which sterically prevent the entrance of MeIm

Table I. Catalase Activity of Manganese Porphyrins[a]

Mn porphyrin	[MeIm],[b] M	O_2 evolution initial rate, mol min^{-1}	turnover rate, min^{-1}
1	0	0	0
	7.5×10^{-3}	6.2×10^{-6}	10.3
	7.5×10^{-2}	5.4×10^{-5}	90.0
	1.5×10^{-1}	7.5×10^{-5}	125
2a	1.5×10^{-1}	0	0
2b	1.5×10^{-1}	1.8×10^{-4}	325
3	0	0	0
	1.5×10^{-2}	0	0
Mn(TPP)Cl[c]	0	2.4×10^{-7}	0.4
	1.5×10^{-2}	4.0×10^{-6}	1.6
4	0	2.4×10^{-7}	0.40
	1.9×10^{-1}	8.4×10^{-7}	1.40

[a] Conditions: [Mn porphyrin] = 3.75×10^{-4} M (as a porphyrin monomer), $[H_2O_2] = 6.96 \times 10^{-2}$ M in an acetonitrile solution (1.60 mL), $T = 10.0 \pm 0.2$ °C. [b] 1-Methylimidazole. [c] TPP: *meso*-tetraphenylporphyrin.

(1) For recent reviews: Wieghardt, K. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 1153-1172. Christou, G. *Acc. Chem. Res.* **1989**, *22*, 328-335.

(2) Dismukes, G. C. *Photochem. Photobiol.* **1986**, *43*, 99-115. Brudvig, G. W. *J. Bioenerg. Biomembr.* **1987**, *19*, 91-142. Pecoraro, V. *Photochem. Photobiol.* **1988**, *48*, 249-264.

(3) *Lactobacillus plantarum*: (a) Beyer, W. F., Jr.; Fridovich, I. *Biochemistry* **1985**, *24*, 6460-6467. (b) Kono, Y.; Fridovich, I. *J. Biol. Chem.* **1983**, *258*, 13646-13648. *Thermus thermophilus*: (c) Allgood, G. S.; Perry, J. J. *J. Bacteriol.* **1986**, *168*, 563-567. (d) Barynin, V. V.; Vagin, A. A.; Melik-Adamyan, V. R.; Grebenko, A. I.; Khangulov, S. V.; Popov, A. N.; Andrianova, M. E.; Vainshtein, A. B. K. *Sov. Phys.—Dokl.* **1986**, *31*, 457-459. (e) Khangulov, S. V.; Voyevodskaya, N. V.; Varynin, V. V.; Grebenko, A. I.; Melik-Adamyran, V. R. *Biophysics* **1987**, *32*, 1044-1051. (f) Khangulov, S. V.; Andreeva, N. E.; Gerasimenko, V. V.; Gol'dfel'd, M. G.; Barynin, V. V.; Grebenko, A. I. *Zh. Fiz. Khim.* **1990**, *64*, 17-27; *Russ. J. Phys. Chem.* **1990**, *64*, 10-16.

(4) Frash, W. D.; Mei, R. *Biochim. Biophys. Acta* **1987**, *891*, 8-14. Wydrzynski, T.; Mano, J.; Takahashi, M.; Asada, K. *Biochemistry* **1987**, *26*, 2495-2501. Ångström, J.; Vänngård, T. *Biochim. Biophys. Acta* **1989**, 973, 23-28.

(5) Mathur, P.; Crowder, M.; Dismukes, G. C. *J. Am. Chem. Soc.* **1987**, *109*, 5227-5233.

(6) Anthracene- and biphenylene-linked porphyrin dimers **1-3** were synthesized by modification of the reported method. Cf.: Chang, C. K.; Abdalmuhdi, I. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 164-165.

(7) Formation constants of mono(imidazole) (K_1) and bis(imidazole) complexes(β) at 20 °C: **1**, $K_1 = 1.2 \times 10^4$ M^{-1}, $\beta = 0$ M^{-1}; **4**, $K_1 = 1.6 \times 10^4$ M^{-1}, $\beta = 1.36 \times 10^7$ M^{-1}.

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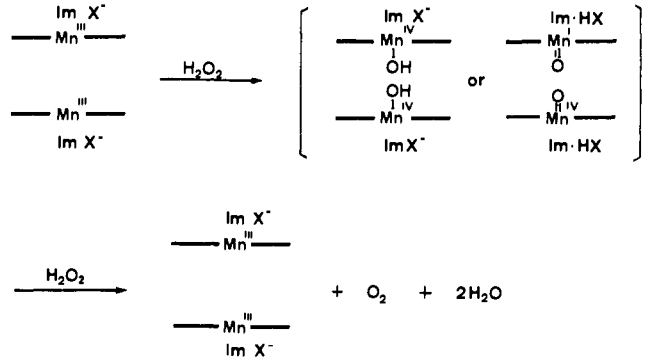
Scheme I



into the cavity. The role of imidazole in H_2O_2–Mn porphyrin systems is reported to be both the acceleration of O–O bond homolysis and the stabilization of an intermediate $Mn^{IV}(=O)$ complex.[8] In marked contrast with **1** and **2b**, biphenylene-linked porphyrin **3** had absolutely no catalase activity even though the predicted metal–metal distance (3.8 Å)[9] is similar to that of the Mn-CAT from *T. thermophilus* (3.6 Å).[3d] This could be explained by formation of the inert μ-oxo dimer, since, after treatment of the reaction mixture of **3** with H_2O_2, the corresponding intramolecular μ-oxo complex was detected by means of FABMS.[10] This had absolutely no catalase activity.

The binuclear center is essential for catalysis, as seen by compounds **2a** and **2b**. The monomanganese complex **2a** has absolutely no catalytic activity. Spectrophotometric analysis[7] revealed that a bis(imidazole) Mn complex did not form, thus confirming that the second Mn porphyrin in the dimer serves to sterically block imidazole ligation to the first one. From these results, two Mn ions are essential and the intermetal distance is important for the development of high catalase activity. In the anthracene-linked porphyrin dimer, this is around 4.5 Å,[9] which is rather longer than the enzymatic one. On the other hand, the turnover rate for *T. thermophilus* enzymes is much larger ($k \approx 10^7$ s^{-1}).[3f]

For clarification of the oxygen evolution mechanism, we used isotopically labeled hydrogen peroxide and analyzed the evolved oxygen by mass spectrometry.[11] When a 1:1 molar ratio of $H_2{}^{16}O_2$–$H_2{}^{18}O_2$ was used, the evolved oxygen was also in a 1:1 molar ratio of $^{16}O_2$ and $^{18}O_2$ and with no ^{16}O–^{18}O detected in the initial stage of the reaction. Furthermore, by kinetic measurement, the initial rate of O_2 evolution was observed to be first order in [**1**] and [H_2O_2]. Thus O_2 evolution proceeds unimolecularly in H_2O_2 at the rate-determining step. Since it has been established that rapid formation of the Mn=O complex occurs by treatment of Mn porphyrins with H_2O_2 in the presence of imidazole,[12] the Mn porphyrin dimer is very likely to form the corresponding bis Mn=O complex, which can reductively decompose and evolve oxygen in unimolecular fashion. This mechanism is also supported by the in situ formation of Mn=O porphyrin dimers and their succeeding rapid reaction with H_2O_2.[13] Thus, we propose a new mechanism for the decomposition of H_2O_2 (Scheme I).

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Supplementary Material Available: Mass spectral charts of evolved oxygen and kinetic data (2 pages). Ordering information is given on any current masthead page.

(8) Balasubramanian, P. N.; Schmidt, E. S.; Bruce, T. C. *J. Am. Chem. Soc.* **1987**, *109*, 7865-7875.

(9) Fillers, J. P.; Ravichanran, K. G.; Abdalmuhdi, I.; Tulinsky, A.; Chang, C. K. *J. Am. Chem. Soc.* **1986**, *108*, 417-424.

(10) After treatment of **3** with H_2O_2, the residual porphyrin showed m/z = 1452 ($M + 2H^+$), 1450 (M^+), instead of the starting porphyrin dimer, m/z = 1435 ($M - 2Cl^- + H^+$). The observed new peaks are assigned to the corresponding intramolecular μ-oxo complex, which regenerates **1** by treatment with a dilute HCl solution.

(11) A direct-inlet apparatus from a reaction cell to the mass spectrometer was used for the analysis of evolved O_2. Cf.: Hoch, G.; Kok, B. *Arch. Biochem. Biophys.* **1963**, *101*, 160-170.

(12) Balasubramanian, P. N.; Schmidt, E. S.; Bruce, T. C. *J. Am. Chem. Soc.* **1987**, *109*, 7865-7873.

(13) Treatment of **2b** in CH_2Cl_2 with *m*-chloroperbenzoic acid (mCPBA) at -30 °C in the presence of MeIm gave the corresponding Mn=O porphyrin dimer (cf. ref 14). Addition of H_2O_2 to the resultant solution showed the instantaneous disappearance of the Mn=O complex (403 nm) and simultaneous regeneration of the Mn^{III} complex (463 nm). A similar operation using manganese *meso*-tetramesitylporphyrin indicated only slow disappearance of the Mn=O complex even in the presence of an excess amount of H_2O_2. For efficient O_2 evolution from H_2O_2, two high-valent Mn ions are essential.

(14) Groves, J. T.; Stern, M. K. *J. Am. Chem. Soc.* **1988**, *110*, 8626-8638.

Proton NMR Spectra without Spin–Spin Splittings

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Complete homonuclear decoupling of NMR spectra has been a long-sought goal[1-5] since it promises direct observation of chemical shifts without the overlap problems associated with spin–spin splitting. We propose a new variation of two-dimensional spectroscopy that displays only proton chemical shifts in one frequency dimension and separated spin multiplet structures in the other. It is an extension of the method of *J*-spectroscopy[6] which calculates the Fourier transform of the spin-echo modulation[7] observed in homonuclear coupled spin systems. Previous approaches to this problem have been complicated by severe distortions of the line intensities[1-3] or very unfavorable line shapes.[8] We show that a one-dimensional spectrum may be recorded which consists only of singlet responses at the chemical shift frequencies, with no fine structure due to proton–proton splittings. These resonances are in the pure absorption mode with intensities proportional to the number of equivalent protons at each site. This mode of display is analogous to the usual practice for natural-abundance carbon-13 spectra recorded with broadband proton decoupling.

We modify the usual spin-echo pulse sequence[1,6] to include a 30-ms adiabatic pulse[9] followed by a 90° pulse at the end of the evolution period. This destroys certain components of the echo modulation by dispersing the corresponding spin isochromats in the spatial inhomogeneity of the radio-frequency field. This alters the character of the spin multiplet structure and permits a pure absorption-mode presentation. We use symmetry[10,11] to simplify the two-dimensional spectrum. There is a plane of symmetry through the $F_1 = 0$ axis and each spin multiplet pattern possesses local C_4 symmetry,[11] making it possible to employ a "symmetry filter" which suppresses all signals lacking a C_4 rotation axis. In practice two consecutive local symmetrizations are performed (with respect to the 45° and 135° diagonals) by examining intensities at symmetrically related frequency coordinates and substituting

(1) Aue, W. P.; Karhan, J.; Ernst, R. R. *J. Chem. Phys.* **1976**, *64*, 4226-4227.

(2) Bax, A.; Freeman, R.; Morris, G. A. *J. Magn. Reson.* **1981**, *43*, 333-338.

(3) Bax, A.; Freeman, R. *J. Magn. Reson.* **1981**, *44*, 542-561.

(4) Shaka, A. J.; Keeler, J.; Freeman, R. *J. Magn. Reson.* **1984**, *56*, 294-313.

(5) Sørensen, O. W.; Griesinger, C.; Ernst, R. R. *J. Am. Chem. Soc.* **1985**, *107*, 7778-7779.

(6) Freeman, R.; Hill, H. D. W. *J. Chem. Phys.* **1971**, *54*, 301-313.

(7) Hahn, E. L.; Maxwell, D. E. *Phys. Rev.* **1952**, *88*, 1070-1084.

(8) Bodenhausen, G.; Freeman, R.; Niedermeyer, R.; Turner, D. L. *J. Magn. Reson.* **1977**, *26*, 133-164.

(9) Titman, J.; Davis, A. L.; Laue, E. D.; Keeler, J. *J. Magn. Reson.* **1990**, *89*, 176-183.

(10) Pfändler, P.; Bodenhausen, G. *J. Magn. Reson.* **1988**, *79*, 99-123.

(11) Boentges, S.; Meier, B. U.; Griesinger, C.; Ernst, R. R. *J. Magn. Reson.* **1989**, *85*, 337-358.

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