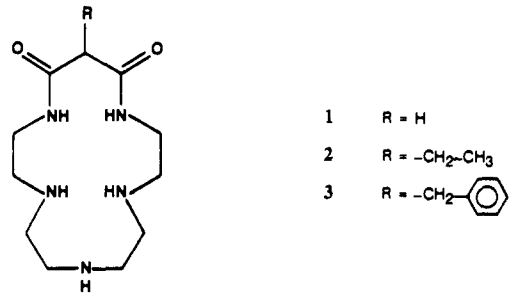
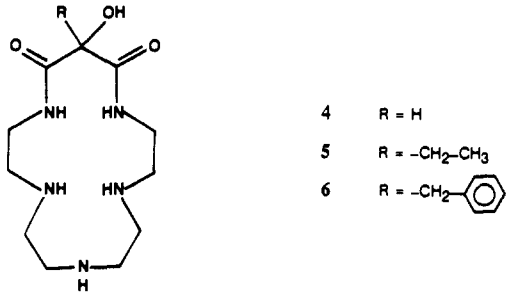


1 R = H

2 R = -CH_2-CH_3

3 R = -CH_2-



4 R = H

5 R = -CH_2-CH_3

6 R = -CH_2-

from Cu(I) complexes. Also, Kimura et al.[5,6] has reported hydroxylation of benzene, toluene, and anisole by nickel–dioxygen complexes.

Ligands **1–3** were synthesized by a modification of the method of Kimura et al.[7] and were characterized by ^{1}H NMR, mass spectra, and melting point. A detailed thermodynamic study ($\Delta G°$, $\Delta H°$, $\Delta S°$) of the formation of the 1:1 dioxygen complexes of the Ni(II) complexes of **1–3** will be published elsewhere.[8]

In order to identify the products resulting from the attack by activated dioxygen on the ligands of the nickel–dioxygen complexes, product analysis was carried out as follows. At 25.0 °C, 500 mg of the ligand and equimolar $NiCl_2 \cdot 6H_2O$ were added to 100 mL of 0.10 M borax buffer solution (pH 10.3) under anaerobic conditions to form the pale purple complexes ($NiH_{-2}L$), where L = **1**, **2**, or **3** and two amide hydrogen ions are displaced from the ligand. Air or dioxygen was then bubbled through the solution for 24–48 h, producing color changes from the initial pale purple to deep brown, and finally to yellow. A 2.0-g sample of solid ethylenediaminetetraacetic acid was added to the reaction mixture, with stirring, whereupon the pH dropped to 3.5. The pH was increased to 11 by addition of 50% NaOH solution, and the color changed to green (Ni(II)–EDTA). About 90% of the solvent water was removed by evaporation, and the residual solution was extracted three times with chloroform. The oxidized ligand was obtained as a white solid by evaporation of the chloroform solvent and was analyzed by ^{13}C NMR. The spectra of all three ligands had new strong resonances at 78.6, 80.5 and 87.7 ppm (relative to TMS) for **4–6**, respectively. These peaks are assigned to the group RCOH, where R = H, ethyl, and benzyl, respectively. For **4**, the ^{1}H NMR showed a new single resonance at 4.5 ppm, which disappeared after addition of D_2O, so that it represents the active H of RCOH. Mass spectra of the oxidized ligand gave the molecular ions 363 (**6**), 301 (**5**), and 273 (**4**), all of which are 16 mass units above those of the original ligands. These data clearly indicate the insertion of an oxygen atom in each of these ligands. NMR studies of the reaction products showed the yields to be 85 ± 5%, based on the amount of macrocyclic ligand employed.

In order to determine the source of oxygen in the insertion reaction, degradation product analysis was carried out on the Ni(II) complex of **3** in the same manner as the $^{16}O_2$ oxygenation products, but with $^{18}O_2$ as the only source of dioxygen. The mass spectral analysis gave a molecular weight of 365 (rather than 363 for the $^{16}O_2$ reaction), definitely demonstrating that the inserted oxygen came from the gaseous $^{18}O_2$ via the dioxygen complex.

The degradation rates of the three Ni(II)–dioxygen complexes are significantly different. For the Ni(II)–dioxygen complex of **1**, the dark brown color characteristic of the dioxygen complex lasted several (5–10) min. For the corresponding complex derived from **2**, it persisted for about 1 h, while for **3** it lasted 4 h. Thus the rates of degradation of the dioxygen complexes varied in the order **1** > **2** > **3**. This difference is ascribed to the protective and stabilizing effects of the alkyl and aralkyl groups on the corresponding dioxygen complexes. The Ni(II) complexes of the hydroxylated ligands showed no tendency to combine with dioxygen. It is suggested that the highly polar hydrophilic hydroxyl group in the ligand has a destabilizing effect on coordinated superoxide. This seems to be in accord with the stabilizing effect observed when the ligand is substituted at the same position by a hydrophobic alkyl or aralkyl group (as in **2** and **3**).

The high yields of hydroxylation products **4–6** indicate that oxygen insertion into the ligand is the main reaction pathway for oxygen activation in these nickel–dioxygen complexes. Although Kimura[5,6] found a loss of 40% of the nickel(II)–dioxygen complex in conversion of benzene to phenol under similar reaction conditions, the quantities of benzene employed and of phenol found were not reported. Because much or most of the loss of dioxygen complex may now be ascribed to ligand hydroxylation, there can be no doubt that aromatic hydroxylation is a minor hydroxylation pathway under the conditions employed (i.e., in aqueous solution) and that nearly all of the dioxygen activation is directed toward ligand hydroxylation. These results do not preclude, however, the possibility that aromatic substrate hydroxylation by these nickel(II) dioxygen complexes may turn out to be a major dioxygen activation pathway under entirely different reaction conditions (i.e., in aprotic low dielectric constant solvents).

Acknowledgment. This work was supported by a research contract with L'Air Liquide, S.A., Paris, France.

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Importance of Hydrogen Bond Acceptor Ability in Design of Host Molecules Capable of Molecular Recognition

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Received February 23, 1990
Revised Manuscript Received October 13, 1990

Use of pyridine units as hydrogen-bond (H-bond) acceptors in the design of host molecules is of current interest.[1-3] We report here that decreasing the pyridine basicity in hosts **1** can destroy the ability to complex H-bond, donating phenols (ρ -2.3).[4] From the molecular architecture perspective, the host–guest K_{assoc}

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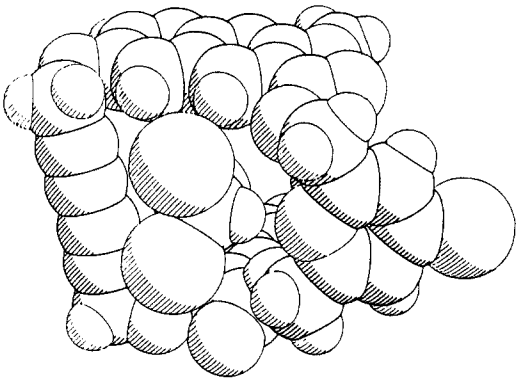
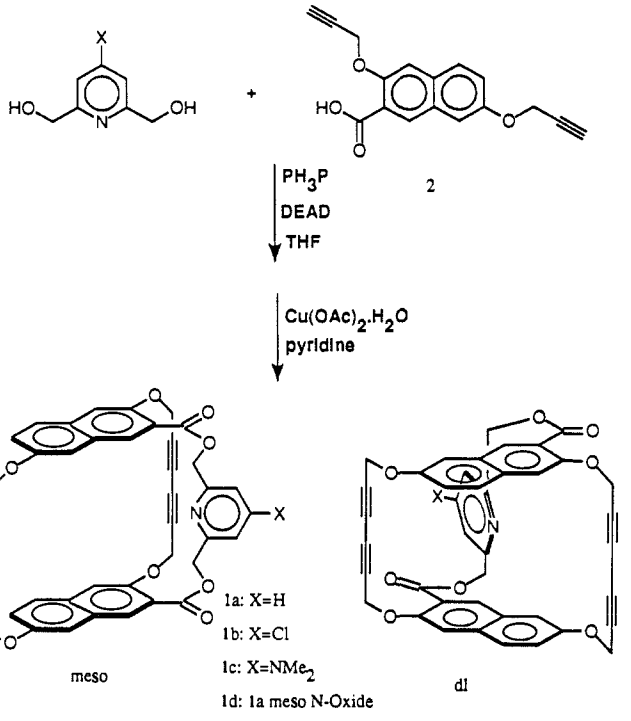


Figure 1. Space-filling representation of the X-ray structure of *dl*-**1b**. The $CHCl_3$ inside the cavity has been omitted. The N–C(chloroform) distance is 3.24 Å.

Scheme I



sensitivity to pyridine basicity makes the difference between very good (e.g., **1c**) and poor (**1b**) hosts. The enthalpic contribution of the H-bond thus is of importance, even though the presence of the highly organized cavity is also required.[3a] The point we wish to make in this paper is that *both* the H-bond and the organized cavity are of crucial importance as design criteria for this type of host molecule.

Cyclophanes **1a** and **1b** (Scheme I) were synthesized in two steps from acid **2** and the appropriate pyridine diol.[3d] In each case meso and *dl* isomers were separated by fractional recrystallization and their identities established by comparison of their NMR and UV spectra with those of **1c**.[5] Assignment of structure was confirmed by X-ray crystal analysis of the major isomer of **1b**, shown to be the *dl* sterioisomer (Figure 1). Oxidation (MCPBA) of **1a** afforded the efficient host *N*-oxide **1d**, shown to be meso by X-ray analysis (Figure 2).

Conclusions. Hosts **1** form intracavity complexes[6] with a variety of phenols in $CDCl_3$ (Table I).[3b,c,7] Several points may be made:

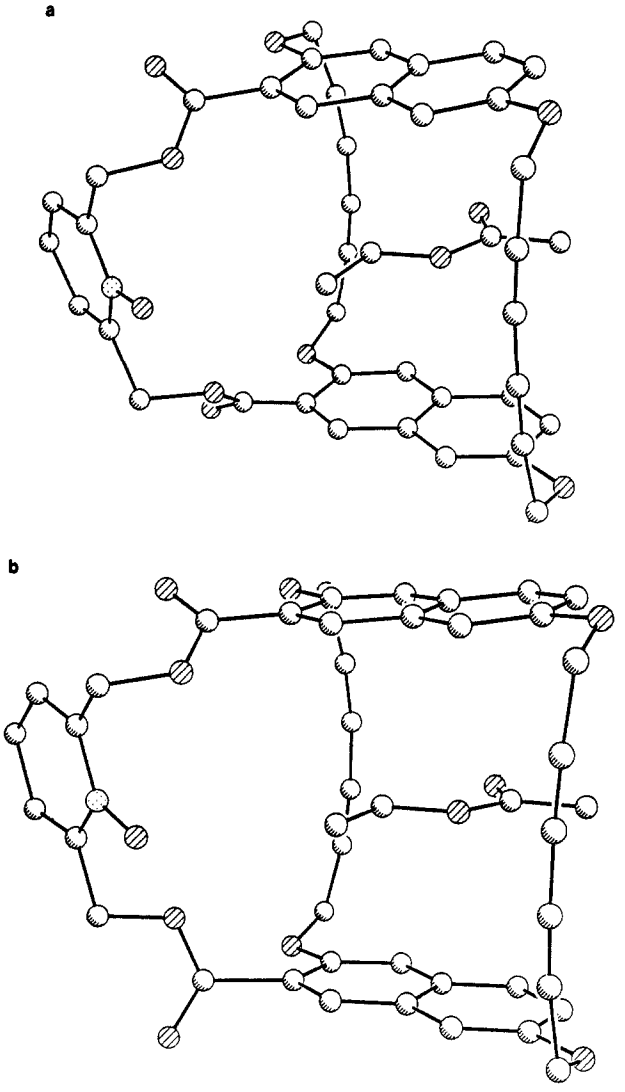


Figure 2. X-ray structure of **1d** *N*-oxide. Both conformations are shown. Occupancies in the crystal lattice: (a) 0.52, (b) 0.48. The hydrogens have been omitted. The ethoxy methyl carbon (of ethyl acetate) to *N*-oxide oxygen distance is 3.50 Å.

Table I. Complexation of **1a** and **1b** in Chloroform

guest	K_{assoc}, M^{-1}			
	meso-**1a**	*dl*-**1a**	*meso*-**1b**	*dl*-**1b**
4-nitrophenol	2340	1156	700	207
4-cyanophenol	815	244	321	94
3-nitrophenol	115	142	52	19
6-nitro-2-naphthol	208			
4-propoxybenzoic acid	8		7	8

Table II. Comparison of Complexation Ability for Series **1** in Chloroform. Guest = 4-Nitrophenol

host	K_{assoc}, M^{-1}	
	meso	dl
1a	2340	1156
1b	700	207
1c	16000	6003
1d	11000	

(1) In all cases the meso host forms more stable complexes than the *dl*. (2) Complex stability decreases sharply as the pyridine

(5) Satisfactory ^1H NMR, IR, and mass spectral data have been obtained for all intermediate compounds. Hosts **1a** and **1b** were characterized by ^1H NMR, IR, and FAB-MS.

(6) (a) A program NLSQ was written using the SIMPLEX algorithm according to ref 6b. Program NLSQ handles several complexation cases and has an attached PostScript and graphical user interface that produces plots interactively. The source (Turbo C) is available from the authors on request. (b) Noggle, J. H. *Physical Chemistry on a Microcomputer*; Little, Brown & Co.: Boston, 1985; pp 145–165.

(7) For association constants for phenol with pyridines, see: Hopkins, H. P., Jr.; Alexander, C. J.; Ali, S. Z. *J. Phys. Chem.* **1978**, *82*, 1268. For association constants for pyridine with phenols, see: (a) Pilyugin, V. S.; Vasin, S. V.; Zinov'ev, V. V. *Zh. Obshch. Khim.* **1984**, *54*, 684. (b) Pilyugin, V. S.; Vasin, S. V.; Maslova, T. A. *Zh. Obshch. Khim.* **1981**, *51*, 1460. (c) Pilyugin, V. S.; Vasin, S. V. *Zh. Obshch. Khim.* **1979**, *49*, 2056.

H-bond acceptor becomes less basic (Table II). A ρ of $\sim$-2.3 may be calculated.[8] With the guest *p*-nitrophenol, complex stability (in $CDCl_3$) decreases approximately 2 orders of magnitude from best to poorest host (Table II). (3) The basic but geometrically quite distinct *N*-oxide is an effective host.

We tentatively relegate the role of the cavity in this series of hosts to the crucial but curiously passive one of immobilizing the guest. A detailed factoring of the roles of cavity and binding site(s) awaits further work.[9]

Acknowledgment. We thank Dr. Douglas R. Powell for obtaining the crystal structures of **1d** and *dl*-**1b**.

Supplementary Material Available: Tables of crystallographic data collection, atom coordinates, and bond distances and angles for **1d** and *dl*-**1b**, *R* values (errors) for the association constants, and synthetic schemes (31 pages); tables of observed and calculated structure factors for **1d** and *dl*-**1b** (32 pages). Ordering information is given on any current masthead page.

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Peroxidase Activity of an Antibody-Heme Complex

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Received August 7, 1990

The specificity and diversity of the immune system have recently been exploited in the generation of antibodies that catalyze a wide variety of chemical reactions.[1,2] Several general strategies for the design of catalytic antibodies have emerged, including the use of antibody binding energy to enhance the chemical reactivity of a cofactor or to position a cofactor and a substrate in close proximity.[3,4] An intriguing target for antibody-cofactor catalysis is the oxidative reactions characteristic of heme proteins. Here we report that antibodies specific for *N*-methylmesoporphyrin IX bind iron(III) mesoporphyrin IX and that the resulting complex catalyzes the oxidation of several substrates. These studies are a first step toward the development of selective antibody-heme monooxygenase catalysts.

Horseradish peroxidase (HRP) is an exceptionally well studied heme enzyme that catalyzes the two-electron reduction of hydrogen peroxide and alkyl hydroperoxides by a variety of electron donors.[5,6] Iron(III) porphyrins and several nonperoxidative heme proteins catalyze peroxidation reactions and have been studied as models for HRP.[7-10] In the course of a separate investigation,[11] we prepared monoclonal antibodies specific for *N*-methylmesoporphyrin IX (**1**), a presumed transition-state analogue for porphyrin metalation. Two of three antibodies obtained catalyzed the chelation of a variety of metal ions by mesoporphyrin IX (**2**). Further study of the faster of the two antibodies, 7G12-A10-G1-A12, revealed that iron(III) mesoporphyrin (**3**) and manganese(III) mesoporphyrin effectively inhibited antibody catalysis. This evidence for the formation of a specific, stable complex of the antibody with an iron porphyrin led us to investigate the oxidative chemistry of the complex.

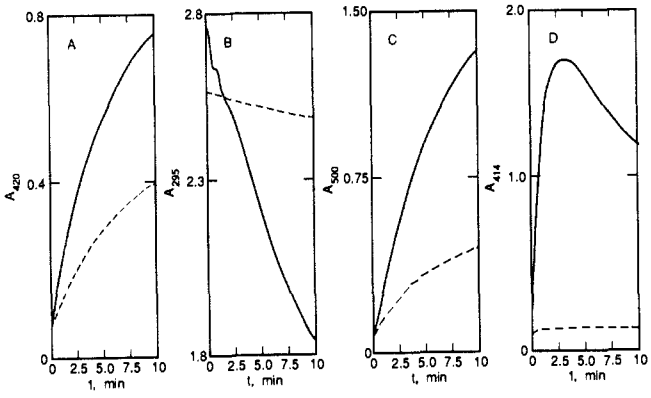

Figure 1. Peroxidation of the indicated substrates in the presence of antibody-mesohemin complex (—) and mesohemin (---). (A) pyrogallol (**4**), (B) hydroquinone (**5**), (C) *o*-dianisidine (**6**), (D) ABTS (**7**).

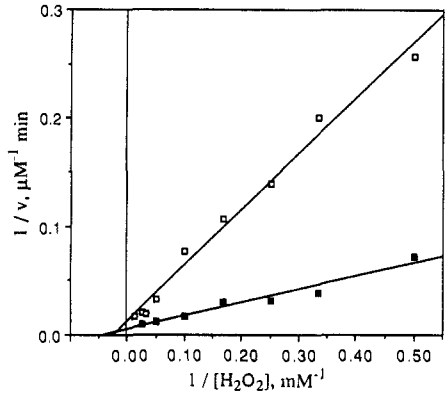

Figure 2. Lineweaver-Burk plots for peroxidation of 0.5 mM **6** as a function of $[H_2O_2]$ for mesohemin- (□) and antibody-mesohemin- (■) catalyzed reactions. Other reaction conditions were as described above.[12]

The antibody·**3** complex was found to catalyze the reduction of hydrogen peroxide by several typical chromogenic peroxidase substrates: pyrogallol (**4**), hydroquinone (**5**), *o*-dianisidine (**6**), and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, **7**). Absorbance versus time curves for iron(III) mesoporphyrin catalyzed peroxidation with and without excess antibody 7G12-A10-G1-A12 are shown in Figure 1.[12] In all cases, peroxidation catalyzed by the antibody·**3** complex is faster than oxidation in the presence of **3** alone. The data in Figure 1 indicate approximately 200-500 turnovers by the antibody·**3** complex in the

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(12) Reaction mixtures contained 1 mM reducing substrate, 5 mM hydrogen peroxide, 0.5 μM iron(III)mesoporphyrin chloride, 0.5% (w/v) Triton X-100, 4% (v/v) dimethyl sulfoxide (DMSO), and 90 mM Tris acetate (pH 8.0) were incubated at 10 °C. Antibody samples contained 0.2 mg/mL (1.3 μM) protein. Reactions were monitored at the indicated wavelengths with a Kontron Instruments Uvikon 860 UV-visible spectrophotometer. $\Delta\epsilon$ values are 3200,[7] -2500,[8] 7500 (approximate),[13] and 36 000 M^{-1} cm^{-1} [14] for the oxidation of **4**-**7**, respectively. One consequence of the reaction conditions employed here is noteworthy. The peroxidation of the two phenolic substrates **4** and **5** by free mesohemin (**3**) was completely suppressed by the added Triton X-100 or by Tween 20 (added to prevent hemin dimerization). In contrast, peroxidation of **6** by **3** was slightly stimulated by the added detergent; this peroxidation was the only one of the four shown that was catalyzed by **3**. The addition of detergent had no effect on oxidation of **7** in the presence of **3** or on any of the antibody-catalyzed peroxidations.

(13) The given value is taken from a commercial assay for peroxidase activity performed in acetate buffer at pH 5.1 (Sigma Chemical Co.) and thus may not be absolutely correct for the reaction conditions used here.

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