H-bond acceptor becomes less basic (Table II). A ρ of ~ -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.

(8) For protonation by *p*-toluenesulfonic acid in $CHCl_3$ we get a ρ of approximately -6. See: Davis, M. M. Bronsted Acid-Base Effects in "Inert" Organic Solvents. In *The Chemistry of Nonaqueous Solvents*; Lagowski, J. J., Ed.; Academic Press: New York, 1970; Vol. 3, Chapter 1.

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Peroxidase Activity of an Antibody-Heme Complex

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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.

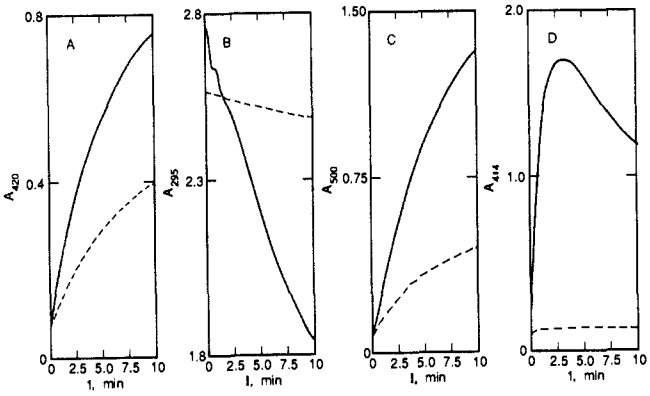

Figure 1. Peroxidation of the indicated substrates in the presence of antibody-mesohemin complex (—) and mesohemin (---). (A) pyrogallol (**4**), (B) hydroquinone (**5**), (C) *o*-dianisidine (**6**), (D) ABTS (**7**).

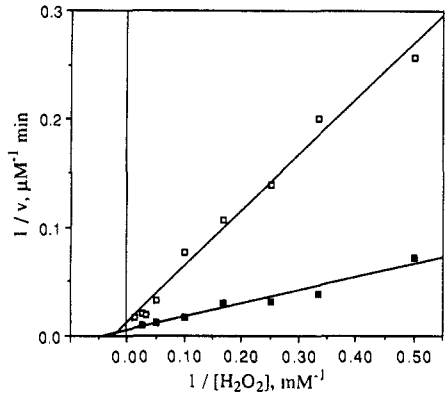

Figure 2. Lineweaver-Burk plots for peroxidation of 0.5 mM **6** as a function of $[H_2O_2]$ for mesohemin- (□) and antibody-mesohemin- (■) catalyzed reactions. Other reaction conditions were as described above.[12]

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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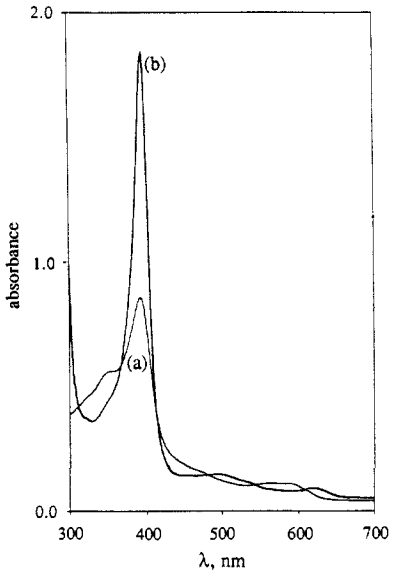

Figure 3. Visible spectra of free mesohemin (a), and the antibody-mesohemin complex (b). Both samples contained 10 μM iron(III) mesoporphyrin, 5% (v/v) DMSO, 0.5% (w/v) Triton X-100, and 95 mM Tris acetate (pH 8.0). The antibody sample contained 3 mg/mL (20 μM) protein.

10-min reaction period. Addition of a stoichiometric amount (relative to antibody binding sites) of hapten **1** completely blocked antibody catalysis. Antibody alone had no peroxidase activity. The other two antibodies specific for hapten **1** did not form mesohemin complexes with peroxidase activity.

The rate of *o*-dianisidine (**6**) oxidation was examined at several *o*-dianisidine concentrations. Catalysis by the antibody·**3** complex reaches a maximum at approximately 0.5 mM **6**, possibly due to competition for the porphyrin binding site by the aromatic substate at higher concentrations. We have not yet determined whether binding of **6** to the antibody contributes to catalysis; however, the wide range of acceptable reducing substrates in this system suggests that a specific binding site for **6** is unlikely. Hydrogen peroxide dependence was therefore investigated at the maximum, 0.5 mM **6**.

The peroxidation reaction displayed saturation kinetics with respect to hydrogen peroxide. Lineweaver-Burk plots for both free iron(III) mesoporphyrin and its antibody complex are shown in Figure 2 (Ig·**3**: K_m = 24 mM, k_{cal} = 394 min^{-1}. **3**: K_m = 43 mM, k_{cal} = 166 min^{-1}). Peroxidases are among the most efficient enzymes known, with $k_{cal}/K_{m(H_2O_2)}$ values of approximately 10^7 M^{-1} s^{-1}. The corresponding value for the antibody complex is 274 M^{-1} s^{-1} compared to 64 M^{-1} s^{-1} for free mesohemin. The kinetic parameters for antibody-catalyzed peroxidation of the various reducing substrates are comparable: k_{cal}/K_m is 233 M^{-1} s^{-1} for ABTS (**7**) and 122 M^{-1} s^{-1} for pyrogallol (**4**). Peroxidation of these substrates was not catalyzed by free **3**.[12] The peroxidation of substrates **6** and **7** by a nonperoxidative heme protein, sperm whale myoglobin (Fluka), was barely detectable under identical reaction conditions.

The near-UV-visible spectrum of the mesohemin-antibody complex in reaction buffer (Figure 3) shows substantially greater absorbance in the Soret region than the free hemin, consistent with binding in a hydrophobic pocket. The antibody complex has major visible bands at 495 and 620 nm, typical of the spectrum of high-spin ferric heme proteins.[15,16] Free hemin in this case has closely spaced visible bands at 558 and 594 nm, more typical of low-spin ferric porphyrins,[16] presumably due to ligation of iron by Tris buffer. An increase in the pK_a of a coordinated water molecule upon binding to antibody (Fe^{III}–OH versus Fe^{III}–$^+OH_2$) would also explain the observed change in spin state. Although only one of the three antibodies specific for hapten **1** catalyzes mesohemin-dependent peroxidations, both of those catalyzing metal ion incorporation into porphyrin bind **3**, and the resulting complexes yield similar high-spin spectra. It will be of interest to determine whether the antibody contributes any axial ligands to the hemin iron in these complexes.

This work demonstrates that antibody complexes of natural heme cofactors can be prepared, and that these complexes can be expected to participate in many of the chemical processes that distinguish heme enzymes. In addition, eliciting antibodies to porphyrins with other *N*-alkyl groups should lead to catalysts with binding sites for both cofactor and a substrate, perhaps capable of promoting selective oxygen atom transfers.

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A New General Synthesis for Polylithium Organic Compounds

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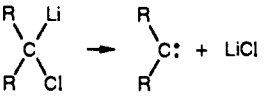
For many years it was thought by organic chemists that it was impossible to prepare polylithium organic compounds.[1] This misconception was changed by work in the early 1970s in our laboratory[2] and in the laboratory of Robert West at the University of Wisconsin.[3] It was perhaps for that reason that the reactions of organolithium reagents with chlorocarbons had not been thoroughly investigated with an eye toward synthesis of polylithium organic compounds.

It is generally believed to be impossible to employ organolithium reagents such as *n*-butyllithium or *tert*-butyllithium to polylithiate multiply halogenated organic compounds, especially if the halogens happen to be on the same carbon or on adjacent carbons. Extremely low yield reactions have been reported when halogens are on opposite ends of a long hydrocarbon chain.[4] The reason that such reaction chemistry is held to be impractical are 2-fold. Molecules having a lithium and a chlorine on the same carbon tend to undergo α lithium halide elimination producing carbenes and lithium halides. Lithium-halogen elimination also occurs



from adjacent carbons. This phenomenon is observed down to temperatures as low as −78 °C, and beyond that there have been few investigations. There is also the important competition between intermolecular coupling reactions of the lithium reagent with the chlorine-substituted species. Both types of reaction chemistry constitute "good" reasons why this reaction chemistry,

(1) Where polylithium compounds are defined as organic compounds containing two or more lithiums on the same carbon or two or more lithium on adjacent carbons. It was widely believed that having two lithiums on the same or adjacent carbon atoms would lead to a destabilization and lithium hydride elimination.
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