Acknowledgment. We thank Scott Farina for the synthesis of the perdeuterio tetramethylthiuram disulfide.

Supplementary Material Available: Tables of atomic coordinates and thermal parameters and full list of bond lengths and bond angles for $Re_2(\mu\text{-}S)_2(S_2CN(C_4H_9)_2)_4\cdot 2OC_4H_8$ (**1b**) and $[Re_2(\mu\text{-}SS_2CN(CH_3)_2)_2(S_2CN(CH_3)_2)_3][SO_3CF_3]\cdot CH_3CN$ (**2a**) (20 pages); full list of observed and calculated structure factors for **1b** and **2a** (30 pages). Ordering information is given on any current masthead page.

Oxidation-State Assignments for Galactose Oxidase Complexes from X-ray Absorption Spectroscopy. Evidence for Cu(II) in the Active Enzyme

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Galactose oxidase, a copper metalloenzyme secreted by the fungus *Dactylium dendroides*, catalyzes the oxidation of primary alcohols to aldehydes and couples this reaction to the reduction of dioxygen to hydrogen peroxide:[1]

$$RCH_2OH + O_2 \rightarrow RCH{=}O + H_2O_2$$

Both the alcohol oxidation and the O_2 reduction half-reactions are two-electron redox processes, yet galactose oxidase contains a single copper atom and no additional metal ions and thus is unusual in its ability to catalyze two-electron redox chemistry at a mononuclear active site. The mechanism of catalysis has remained elusive, in part because of the variability exhibited by the native enzyme prepared according to published procedures.[2] Recently it has become possible to prepare stable, homogeneous and well-defined redox modifications of galactose oxidase for the first time, making available reductively inactivated, oxidatively activated, and a substrate complex as pure forms.[3] This recent work using homogeneous redox forms of galactose oxidase has demonstrated that it is the reductively inactivated enzyme that gives rise to the type 2 Cu EPR signal previously reported for this enzyme, and that this signal is absent in oxidatively activated galactose oxidase, even when redox agents have been removed. This is in agreement with the original report of this perplexing behavior[4] and demonstrates that the active form contains a unique EPR-silent oxidized copper complex.

Several structural proposals have been advanced to account for the unusual two-electron redox activity of galactose oxidase and the distinctive EPR behavior. The first, based on early results, was the plausible proposal of a formal Cu(III) oxidation state involved in a two-electron redox couple: [Cu(III) + $2e^- \leftrightarrow$ Cu(I)].[4] In this model, the reductively inactivated enzyme contains Cu(II), which is oxidized to a formal Cu(III) state on activation, thus accounting for the loss of the EPR signal. A second proposal required that the metal ion be redox inactive, mediating electron transfer between substrate and O_2 while remaining in the cupric oxidation state.[5] According to the third proposal, activation is associated with protein-centered oxidation in which case the oxidatively activated galactose oxidase would contain Cu(II) and a stable organic radical. Spin coupling of two $S = {}^1/_2$ systems could then account for the EPR-silent character of the oxidized enzyme.[3] This proposal is consistent with spectroscopic evidence for Cu(II) in the active enzyme obtained from CD spectroscopy and from a comparison of ligand-to-metal charge-transfer spectra for the N_3^- adducts of oxidatively activated and reductively inactivated galactose oxidase. Recently a fourth model has been proposed in which a pyrroloquinoline quinone cofactor is associated with a Cu(I) active site in the oxidatively activated form of the protein.[6] A definitive assignment of the copper oxidation state in each accessible enzyme form is critical for distinguishing between these structural proposals. We report here X-ray absorption near-edge structure (XANES) spectra for each of the available pure redox modifications of galactose oxidase[11] and the copper oxidation-state assignment derived from each.

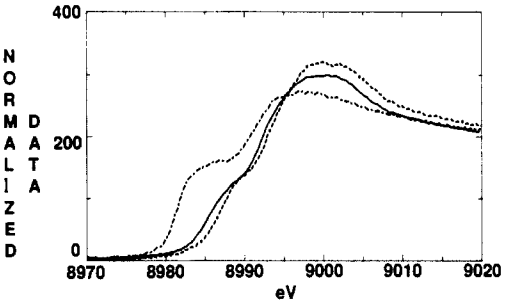

Figure 1. Normalized XANES spectra of oxidatively activated (—), reductively inactivated (- - -), and substrate (-·-) complexes of galactose oxidase. All samples were prepared according to ref 3.

XANES spectra are the structured absorption features that occur over approximately 50 eV in the region of a core-level electronic transition. In general, XANES is sensitive to oxidation state, coordination geometry, ligation type, and bond lengths of the absorbing site, making it a valuable structural probe for metal complexes. Cu(I) XANES spectra are readily distinguished by an intense resolved shoulder at ca. 8984 eV, below the range where more oxidized Cu absorbs. Previous work has demonstrated the utility of XANES, and in particular this Cu(I) feature, in determining Cu oxidation states.[7] Cu(III) XANES spectra are shifted by several electronvolts to higher energy relative to Cu(II),[8] probably due to shorter bond lengths for Cu(III) relative to Cu(II).[9] Blumberg et al. previously reported XANES spectra for galactose oxidase[8] and interpreted these as evidence against the presence of Cu(III) in the oxidatively activated enzyme.

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(10) XANES spectra were measured at the Stanford Synchrotron Radiation Laboratory (SSRL) beam line 4-1 and the National Synchrotron Light Source (NSLS) beam lines X-9A and X11A. Samples were placed in Mylar-windowed Lucite cuvettes at 10 (SSRL) or 77 K (NSLS) and data measured as fluorescence excitation spectra by using a multielement Ge detector array (SSRL) or an ionization chamber (NSLS). Spectra were normalized by subtraction of a polynomial and multiplication by a scale factor so as to give best agreement with tabulated X-ray cross sections (G. S. Waldo, J. E. Penner-Hahn, submitted for publication). X-ray energies were calibrated by reference to a Cu foil (first inflection point of foil 8980.3 eV). Spectra measured under different conditions are identical apart from minor differences in energy resolution [Si(220) monochromator crystals at SSRL vs Si(111) crystals at NSLS].
(11) The different redox states of galactose oxidase were prepared as described in ref 3 and were characterized by their EPR spectra measured on frozen samples in EXAFS sample cells.

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However, these spectra were relatively noisy and subject to concerns regarding sample heterogeneity.

Representative normalized XANES spectra[10] for the three galactose oxidase samples are shown in Figure 1. It is clear that the oxidatively activated and reductively inactivated forms have virtually identical edge regions. As expected, the inactive edge is consistent with Cu(II). The nearly identical appearance of the XANES spectra for reductively inactivated and oxidatively activated galactose oxidase suggests that there is at most a minor change in the Cu site on oxidative activation. In particular, the observation that the edge for the oxidatively activated enzyme is, if anything, at a lower energy than the edge for the inactive protein is *inconsistent* with Cu-centered oxidation. Samples were characterized by EPR spectroscopy and enzyme assay before and after XAS analysis and found to be unchanged. Further, a sample of oxidatively activated enzyme containing an oxidant [hexacyanoferrate(III), 7.5 mM] as a trap gave Cu XANES essentially indistinguishable from the oxidant-free sample, removing the possibility of photoreduction of the sample during irradiation. This provides direct evidence that oxidative activation does not involve metal-centered oxidation. In contrast, the edge structure for the anaerobic substrate complex is a classic example of the XANES spectra typical of Cu(I) complexes,[7] demonstrating that substrate reduces the active-site cupric ion.

The present results provide strong support for a catalytic cycle involving [Cu(II) + enzyme radical] ↔ [Cu(I)] as discussed in ref 3. The XANES spectra suggest some structural differences in the Cu sites for the active and inactive enzyme; however, the nature of the difference is unclear. EXAFS studies, which will permit a more detailed structural characterization of each of these species, are in progress.

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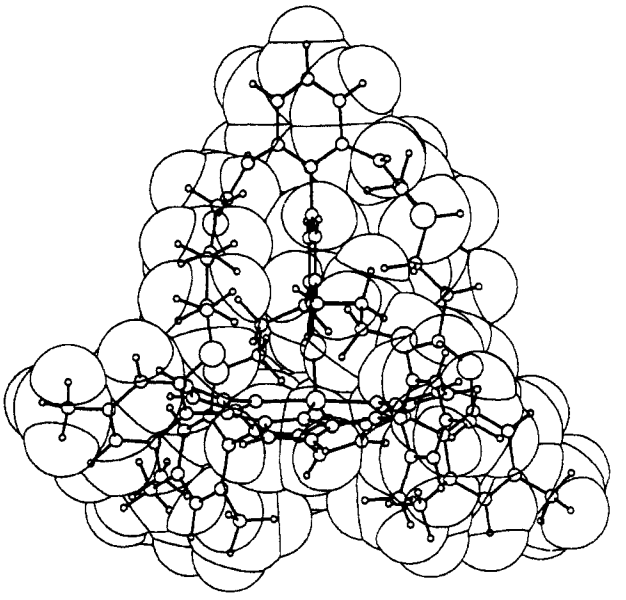

Figure 1. Overlap of "stick and ball" and CPK models of $(IX)Fe^{III}$. The stick and ball model shows the ligation of iron(III) and phenoxide oxygen as well as the role of the biphenyl substructure in supporting the flying bridges that constitute the capping structure. The CPK model shows how the pendant ligand and iron(III) are protected on the capped face. [The coordinates for $(IX)Fe^{III}$ were created by using $CHARM_m$ (Polygen Corp.). The file PORPHYRINH.RTF was used to generate the iron(III) porphyrin ring, and an RTF generated in the 2-D molecular construction routine of Quanta (Polygen, Corp.) was used to generate the cap. The structure was extensively minimized in $CHARM_m$ with steepest descents and adopted basis Newton-Raphson.]

First Synthesis of a Pendant-Capped Porphyrin. A Biphenyl Pendant-Capped Porphyrin Model of Catalase

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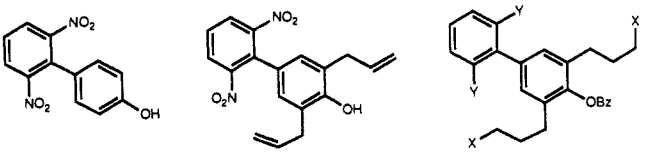
An ideal chemical model for a specific enzyme would include the following structural characteristics: (i) a domain that holds the catalytic functional groups at the active site in a geometry that mimics the active site structure of the protein enzyme; (ii) a binding domain for the substrate; and (iii) a capping structure that protects the catalytic functional groups from unwanted interaction with species other than the substrate. In principle, the structure of (5,10,15,20-tetraphenylporphinato)metal(III) species ($(TPP)M^{III}(X)$) offer a unique opportunity to create the desired features of i–iii in the synthesis of catalase, peroxidase, and cytochrome P-450 models. The ligands (phenolic hydroxyl of tyrosine for catalase, imidazole of histidine for peroxidase, and a sulfhydryl of cysteine for cytochrome P-450) that characterize these enzymes are trans to the reactive side of the porphyrin plane such that the substrate-binding domain and the protective ligating cap are located on opposite faces of the porphyrin. The four phenyl rings of TPP would act as the anchors for the binding domain and protective cap. We report here the first synthesis of a TPP structure with a protective pendant ligand cap.[1]

The identities of all intermediates were determined by ^1H NMR and mass spectroscopy. 4-Methoxy-2′,6′-dinitrobiphenyl (obtained from reaction of 4-iodoanisole with 2,6-dinitrochlorobenzene in the presence of Cu^0 at 230 °C, 90% yield) on demethylation (BBr_3/CH_2Cl_2 at −70 °C, warming to 25 °C for 17 h) provided 4-hydroxy-2′,6′-dinitrobiphenyl I (90%). Reaction of the sodium



I III IV X = OH, Y = NO_2; V X = Br, Y = NH_2

salt of I with excess allyl chloride (20 °C, warming to 70 °C for 18 h) and Claisen rearrangement of the resultant allyl ether (bubbling BCl_3 for 1.5 h in chlorobenzene) provided 3-allyl-4-hydroxy-2′,6′-dinitrobiphenyl (II, 91%). Reaction of the sodium salt of II with allyl chloride and Claisen rearrangement (same procedures as used with the sodium salt of I) gave III (88%). Blocking of the phenolic function of III by benzylation (benzyl bromide with K_2CO_3 in DMF, 25 °C) followed by terminal hydroxylation (9-BBN/THF at 25 °C, warming to 65 °C for 2 h, 3 M NaOH at 5 °C followed by reaction with 30% H_2O_2 at ≤25 °C) provided diol IV (76%). Replacement of the OH groups of IV by Br substituents (Ph_3P/CH_2Cl_2 at 23 °C, collidine at −3 °C, and reaction with CBr_4 at −3 °C) and reduction of the NO_2 substituents ($NH_4Cl/EtOH-H_2O$, Fe^0; 65 °C, 2 h) yielded V

(1) A tailed $(TPP)M^{III}(X)$ possesses a chain of atoms extending from the 2-position of a single phenyl substituent, which terminates in a ligand species. A strapped $(TPP)M^{III}(X)$ possesses a chain of atoms (which holds a ligand in the vicinity of the M^{III} moiety) extending from the 2-position of a phenyl substituent on the 5-meso carbon of TPP to the 2-position of a phenyl substituent on the 15-meso carbon of TPP. Both tailed and strapped structures allow reaction of reagents with the ligand species. This feature is not shared by a pendant-capped porphyrin because the ligand is hung from the ceiling of a vaulted dome or tepee with protective walls supported by four legs that are anchored to the 2-positions of the four phenyl rings at the 5-, 10-, 15-, and 20-meso carbons of the TPP.

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