naphthyl substituent in 8-(β-naphthyl)menthyl crotonate.

The sense of the induction of the adducts in Table I (entries 8-10) could be established by correlation[13] with (*R*)-3-aminobutan-1-ol.[3,14] The results confirm that amine addition does, in fact, take place preferentially on the *re* face of the crotonate unit, in agreement with related work.[11,15]

This new enantioselective route to β-amino esters is incontestably one of the most efficient asymmetric carbon-nitrogen bond-forming reactions known to date; furthermore the new 8-arylmenthols that we have developed during the course of this work will undoubtedly prove to be powerful and general chiral auxiliaries in other enantioselective processes.

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Supplementary Material Available: Preparative methods and spectral data for all new compounds **1** and corresponding amino derivatives **3** (7 pages). Ordering information is given on any current masthead page.

(13) (i) LAH, Et_2O, 20 °C, quantitative; (ii) H_2 3 bar, $Pd(OH)_2$, MeOH, quantitative. The chiral auxiliary alcohols were recovered in 95% yield.

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Oxygenated Cytochrome P-450-CAM and Chloroperoxidase: Direct Evidence for Sulfur Donor Ligation Trans to Dioxygen and Structural Characterization Using EXAFS Spectroscopy

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Cytochrome P-450 and chloroperoxidase are heme iron enzymes that possess unique spectroscopic and catalytic properties.[2,3] The P-450 enzymes activate dioxygen for incorporation into organic molecules while chloroperoxidase couples the reduction of peroxides to the oxidation and incorporation into organic substrates of chloride ions. Despite catalyzing such disparate reactions, extensive spectroscopic studies have consistently shown both enzymes to contain essentially identical heme iron coordination structures.[3]

The P-450 reaction cycle consists of four known intermediates culminating in a ferrous-O_2 adduct (oxy-P-450).[4] One-electron reduction of oxy-P-450 yields the hydroxylated product and water. Through spectral comparison of heme protein and synthetic porphyrin complexes of known structure with isolated P-450 intermediates, cysteinate sulfur has been established as the axial ligand for ferric, deoxyferrous, and ferrous-CO P-450.[2] Recently, the presence of the cysteine ligand in ferric P-450 has been verified by X-ray crystallography.[5] Oxy-P-450 is the last identified intermediate in the reaction cycle and the state about which the least is known. Although replacement of the cysteinate ligand by histidine upon dioxygen binding has been proposed,[6] spectral studies of oxy-P-450 appear to rule out histidine ligation.[7] A thiolate/O_2-ligated ferrous porphyrin complex with Mössbauer properties similar to those of oxy-P-450 has been structurally characterized by Weiss and co-workers.[8]

In contrast to P-450, chloroperoxidase functions without reduction to the ferrous state. Instead, peroxide addition to the ferric enzyme produces an iron-oxo species that reacts with chloride to effect chlorination.[9] Recently, an oxygenated derivative of ferrous chloroperoxidase has been reported[10,11] which, unexpectedly, is spectrally distinct from oxy-P-450-CAM.[10,12] In order to more fully elucidate the structural properties of these enzymes and to further probe the structure/function relationship between P-450 and chloroperoxidase,[3] especially in light of earlier chemical evidence against cysteine ligation in the latter,[13] extended X-ray absorption fine structure (EXAFS) spectroscopy has been used. EXAFS is a particularly useful technique for determining the number, identity, and distance of donor atoms surrounding the central metal in metalloenzymes.[14] In favorable cases, metal-ligand bond distances can be determined in the first coordination shell to an accuracy of ±0.02 Å and coordination number to ±25-35% (±1 atom in 3-4). Here we report the structural characterization of oxygenated P-450-CAM and chloroperoxidase with EXAFS spectroscopy. Evidence is presented that both the enzyme states contain a thiolate sulfur donor axial ligand trans to dioxygen.

The oxygenated derivatives of P-450-CAM (1.4 mM, pH 7.4, in the presence of 4 mM *d*-camphor) and chloroperoxidase (3.5 mM, pH 6.0) were prepared at -40 °C in potassium phosphate buffer containing 65% (v/v) ethylene glycol as described previously.[10] The homogeneity and integrity of the samples before *and* after EXAFS experiments were verified by UV-visible absorption spectroscopy; less than 15% autooxidation occurred during sample manipulation. The spectra were obtained at -80 °C and the

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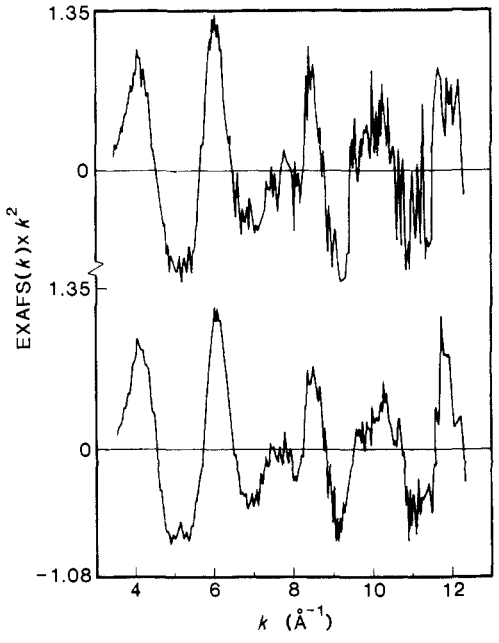

Figure 1. EXAFS spectra of oxygenated cytochrome P-450-CAM at pH 7.4 (top) and oxygenated chloroperoxidase at pH 6.0 (bottom) obtained at −80 °C. A mixed solvent was employed consisting of ethylene glycol [65% (v/v)] and 0.035 M potassium phosphate buffer (plus 4 mM camphor for P-450-CAM). Spectra have been multiplied by k^2 to enhance the visibility of oscillations at high k.

Table I. Structural Comparison of Dioxygen Adducts of Cytochrome P-450-CAM, Chloroperoxidase, and Sulfur Donor Ligated Heme Iron Complexes

	Fe–N (porphyrin)		Fe–S (axial)		Fe–O (dioxygen)	
	bond length, Å	N^a	bond length, Å	N^a	bond length, Å	N^a
oxy-P-450-CAM[b]	2.00	7.8	2.37	1.3	1.78	1.1
oxychloro-peroxidase[b]	2.00	7.4	2.37	1.4	1.77	1.3
thiolate/O_2-ligated heme iron complex[c]	1.990		2.369		1.818	
thioether/O_2-ligated heme iron complex[d]	1.99–2.00		2.49		NR[e]	

[a] The number of ligand atoms. [b] EXAFS data reported in this work. See text for accuracy of bond lengths and N values. [c] Crystallographic data reported in ref 8b by Weiss and co-workers for [(Fe(O_2)-(SC_6HF_4)(TPpivP)]$^-$ [(TPpivP, *meso*-tetra(α,α,α,α-*o*-pivalamidophenyl)porphyrin]. [d] "Semiquantitative" crystallographic data reported in ref 18 by Collman and co-workers for [(Fe(O_2)(THT)(TPpivP)] (THT, tetrahydrothiophene). [e] Not reported.

samples were stored at 77 K when not under study. All X-ray absorption data were collected at the Stanford Synchrotron Radiation Laboratory as fluorescence excitation spectra[15,16] on Beam line II-2 using a Si[220] double-crystal monochromator. The storage ring was operated at 3.0 GeV and 35–60 mA. Analysis of the data was performed as previously reported.[14,17]

The EXAFS spectra of oxy-P-450 and oxychloroperoxidase (Figure 1) are identical to within the noise level of the data. This provides qualitative support for the proposal[10] that the two complexes have identical heme iron coordination spheres and that discrepencies in their UV–visible absorption and magnetic circular dichroism spectra are due to differences in heme iron environments.[3] The bond distances and numbers of atoms for Fe–N-(porphyrin), Fe–S(axial), and Fe–O(dioxygen) obtained by curve-fitting analysis of Fourier-filtered first-shell data for the two oxygenated enzymes are included in Table I, together with analogous crystallographically determined metrical information for thiolate/O_2[8b] and thioether/O_2[18] models. The numbers of ligated atoms obtained from curve-fitting analysis are larger than the expected values (i.e., four for Fe–N and one for Fe–S). This is due to the fact that the protein data, collected at −80 °C, have smaller Debye–Waller factors than the room temperature data for model complexes. As expected for six-coordinate low-spin heme iron species,[19] Fe–N bond distances of 2.00 Å have been determined for both dioxygen adducts, in agreement with previously reported values for the two model complexes.[8b,18] The contribution of the Fe–S backscattering shell is significant as can be seen in Figure 2. The Fe–S bond lengths of 2.37 Å found for both oxygenated enzymes provide quantitative evidence that their coordination structures are identical. The Fe–S bond lengths are equal to that reported for a thiolate/O_2 model[8b] (Table I) and are longer than in the corresponding CO adduct of P-450 (2.32 Å).[20] Lengthening of the Fe–S bond upon replacement of trans CO with O_2 has also been observed in thioether-ligated ferrous porphyrin models (2.41 and 2.49 Å for trans CO[21] and O_2,[18] respectively). A significant improvement in the function value, F,[14,17] of between 23% and 27% occurs after including the Fe–O shell into the fits with just nitrogen and sulfur shells. The Fe–O bond distances for the two protein states agree well with that of the thiolate/O_2 model[8b] (Table I).

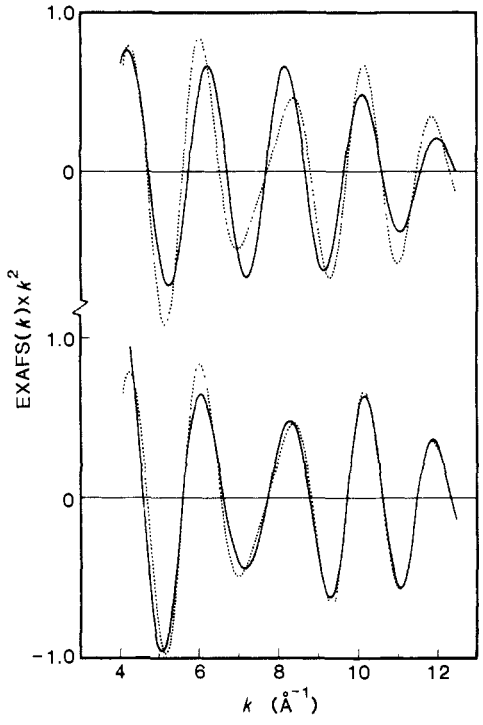

Figure 2. Curve-fitting result for oxy-P-450-CAM. The least-squares fit (solid line) to the first shell filtered data (dot line) without (top) and with (bottom) inclusion of the Fe–S(axial) shell. Fitting was over a range of $k = 4 - 12$ Å^{-1}. The best fits to the data for both oxygenated proteins are qualitatively similar. Numerical results are summarized in Table I.

Having established that both oxygenated enzymes have an axial sulfur donor atom, in order to assign the sulfur donor type (i.e., thiolate, thiol, etc.), it is useful to compare the Fe(II)–S(axial) bond distance with those of structurally related model complexes.

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Unfortunately, in the O_2-bound series, only two examples are known (Table I): with thiolate,[8b] the distance is 2.37 Å; with thioether,[18] it is 2.49 Å. Recently, a series of sulfur-donor-ligated ferrous–CO complexes has been examined with EXAFS spectroscopy[21] and the Fe–S bond length has been found to increase by about 0.1 Å upon going from thiolate (2.32 Å) to any other type (thiol, thioether, or disulfide) of sulfur donor (2.40–2.41 Å). Taken together, the available data suggest that there is an appreciable lengthening of the Fe(II)–S(axial) bond trans to O_2 or CO for nonthiolate relative to thiolate sulfur donors and strongly support the assignment of the sulfur in oxy-P-450 and oxychloroperoxidase as a thiolate type.

In summary, compelling and direct evidence for a sulfur atom, most likely thiolate, as the trans axial ligand in oxy-P-450-CAM and oxychloroperoxidase has been obtained. Iron–sulfur bond distances of 2.37 Å have been determined in both cases. A sulfur donor ligand has now been detected by EXAFS analysis for all of the isolable intermediates in the P-450 reaction cycle and for the ferrous–CO state;[19,21] with chloroperoxidase, this represents the second state where a sulfur donor ligand has been detected by EXAFS.[22] Possible mechanistic implications of having a thiolate ligand in the P-450 system have been discussed.[2,23]

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Differential Anomalous X-ray Scattering Evidence for the Existence of μ-$H_3O_2^-$ Bridging Ligands in Solution

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In aqueous solution, transition-metal aquo ions $M(OH_2)^{n+}$ frequently undergo hydrolysis to μ-hydroxy or μ-oxo bridged dimers and polymers.[2] This reaction is generally presumed[3] to occur via condensation of the primary hydrolysis product $M(OH)^{(n-1)+}$; however, an alternative mechanism[4] involves initial formation of $H_3O_2^-$ bridged species. The $H_3O_2^-$ bridge may also be relevant to electron-transfer reactions, where one mechanism[5] involves H-atom transfer via an $H_3O_2^-$ bridged intermediate. Although recent crystallographic characterization of μ-$H_3O_2^-$ bridged Cr, Mo, and W clusters[4,6,7] offer support for these mechanisms, their significance depends on the extent to which $H_3O_2^-$ bridged species exist in solution. The $H_3O_2^-$ anion has been found in both bridging[4,6,7] and unligated[8] environments, but to date it has been structurally characterized only in crystalline materials. Vapor tensiometry[9] and Mössbauer spectroscopy[10] have provided indirect evidence for μ-$H_3O_2^-$ bridge formation in solutions of Cr and Fe complexes; however, these experiments are neither structurally definitive nor, in the case of the Mössbauer work, necessarily representative of aqueous solutions. We have obtained differential anomalous X-ray scattering (DAS) evidence for the existence of $H_3O_2^-$ bridged species in aqueous solution.

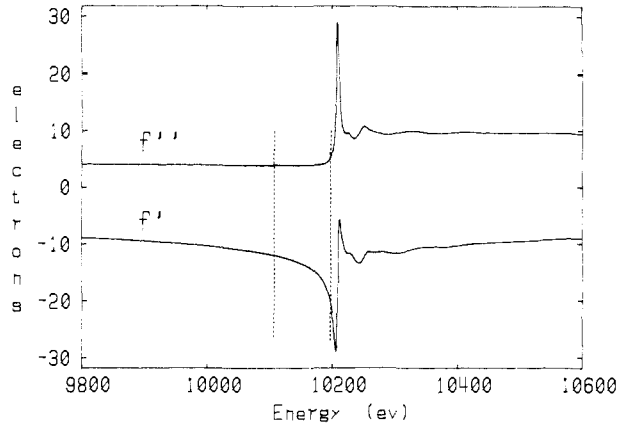

Figure 1. Experimental f_w' and f_w'' values for solution II in the vicinity of the W L_{III} absorption edge. Identical values were observed for solution I. f_w'' values were obtained from absorption measurements by using the optical theorem. f_w' values were calculated from f_w'' data by using the Kramers-Kronig relation. Vertical lines indicate energies for data collection (corresponding to $f_w' = -12.0$ and -20.3 electrons).

The trinuclear cluster $[M_3O_2(O_2CR)_6(H_2O)_3]^{2+}$ (M = W, Mo; R = CH_3, C_2H_5) can be crystallized as a monomer (isolated M_3 clusters) at low pH or as a dimer or polymer at higher pH.[6,7] In the dimer and polymer, partial deprotonation of the water ligands leads to the formation of $H_3O_2^-$ bridges between M_3 clusters. We have used DAS to determine the local atomic environment of the tungstens in two different solutions of W_3 clusters. Solution I was 0.3 M in HCl, thus giving fully protonated (monomeric) W_3 clusters. Solution II contained LiCl in place of HCl, thus favoring dimer and polymer formation.

X-ray scattering is a useful technique for determining the radial distribution function (RDF) of electron density about the average atom in a sample. However, since *all* atomic correlations contribute to the RDF, solute structure determination is extremely difficult for dilute multicomponent solutions. The recent availability of synchrotron radiation, providing a high-flux source of tunable X-rays, has made feasible the development[11] of DAS, in which scattering is measured at two wavelengths.

The X-ray scattering factor $f(k,E)$ for an atom is given by $f(k,E) = f_0(k) + f'(k,E) + if''(k,E)$ where $k = (4\pi \sin\theta)/\lambda$ is the wavevector and E is the incident photon energy. The anom-

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