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Abstract

The iron coordination structures of the oxygenated cytochrome P-450-CAM and chloroperoxidase and of a number of penta- and hexa-coordinate ferrous porphyrin complexes containing biologically relevant, sulfur axial ligands have been investigatied by EXAFS spectroscopy. The two oxygenated enzymes have both been found to contain a sulfur atom located 2.37 Å from the central heme iron. In the CO-ligated series of model complexes, two distinct categories of Fe-S_{ax} distances have been observed that correlate with thiolate and non-thiolate ligation (~2.33 and ~2.41 Å, respectively). The data for these model ferrous-CO ccomplexes, together with previously reported Fe-S distance in oxygenated heme iron model complexes, allows the sulfur atoms in oxygenated P-450-CAM and chloroperoxidase to be identified as a thiolate sulfur.

Introduction

The characterization of iron coordination structure is of considerable importance in understanding the mechanism of action of heme iron enzymes [1]. To that end, metalloporphyrin complexes have been used extensively as both structural and functional models for heme proteins since their ligand composition can be varied to a greater degree than that of the proteins they are intended to mimic. The role of sulfur donor ligation in the function of heme proteins is incompletely understood at present. To date, only a very small number of sulfur donor-ligated ferrous porphyrins have been structurally defined.

Cytochrome P-450 and chloroperoxidase are heme iron enzymes that possess unique spectroscopic and catalytic properties [2,3]. The P-450 enzyme activates dioxygen for incorporation into organic molecules while chloroperoxidase couples the reduction of peroxides to the oxidation and incorporation of chloride ions into organic substrates. Oxy-P-450 is the last identified intermediate in the reaction cycle and the state about which the least is known. Recently, an oxygenated derivative of ferrous chloroperoxidase has been reported [4,5] which, unexpectedly, is spectrally distinct from oxy-P-450-CAM (the soluble camphor-hydroxylating enzyme). 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, EXAFS spectroscopy has been used.

Experimental

Ferrous porphyrin model complexes and oxygenated cytochrome P-450-CAM and chloroperoxidase proteins were prepared and characterized according to published procedures [6,7]. The homogeneity and integrity of the protein samples before and after EXAFS experiments were verified by UV-visible absorption spectroscopy; less than 15% autoxidation occurred during sample manipulation. The protein spectra were obtained at -80°C and the samples were stored at 77 K when not under study. All X-ray absorption data were collected at the Stanford Synchrotron Radiation Labortary as fluorescence excitation spectra on Beam lines II-2 or VII-3 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 [8].

Results and Discussion

The iron coordination structures in a number of penta- and hexa-coordinate ferrous porphyrin complexes containing biologically relevant, sulfur donor axial ligands have been investigated by EXAFS spectroscopy [6]. The curve-fitting results for Fourier-filtered first shell data fits of various sulfur donor-ligated ferrous porphyrin model complexes are given in Table 1. Although some values of N, the number of ligand atoms in a given shell, for S_{ax} are small (Table 1), the contribution of S_{ax} appears significant since in the process of curve fitting analysis, the function value F (which is χ^2 and measures the goodness of fit compared to the experimental data) decreases significantly after including the Fe-S_{ax} shell into the fits. The improvement is between 60-80% for all the cases reported.

EXAFS analysis of the penta-coordinate FeOEP(SPr)$^-$ and hexa-coordinate FeOEP(SPr)(CO)$^-$ complexes in solution showed that the Fe-S_{ax} distance (2.33Å) remained constant, whereas the Fe-N_p distance decreased from 2.05 to 2.00 Å upon CO ligation as expected for the change in coordination number [9]. In contrast, changing the axial ligand from thiolate FeOEP(SPr)(CO)$^-$ to thiol FeOEP(PrSH)(CO) in the hexa-coordinate CO-bound ferrous porphyrin complexes caused a distinct increase in the Fe-S_{ax} distance from 2.33 to 2.41 Å but did not affect the Fe-N_p bond length. Such a large increase in the Fe-S_{ax} bond length (0.08 Å) upon protonation of bound thiolate indicates that the orbital interaction between the heme iron and thiolate sulfur atoms is more extensive. The other hexa-coordinate complexes, FeOEP(THT)(CO) and FeOEP(MeSSMe)(CO), serve as models for methionine and cystine

TABLE 1

Structural Comparisons of Various Sulfur Donor-Ligated Ferrous Porphyrin Complexes and Heme Proteins.

Complex[a]	Fe-N_p R(A)	Fe-N_p N	Fe-S_{ax} R(A)	Fe-S_{ax} N
Five-coordinate:				
FeOEP(SPr)$^-$	2.05	3.8	2.33	0.4
FeTPP(SEt)[b]	2.096	4	2.360	1
Fe^{+2} P-450-CAM	2.08	3.0	2.34-2.38	0.6
Six-coordinate:				
FeOEP(SPr)(CO)$^-$	2.00	4.4	2.33	0.2
FeTPP(SEt)(CO)[b]	1.993	4	2.352, 2.388	1
Fe^{+2}P-450-CAM + CO	1.98	3.3	2.32	1.0
FETpivPP(SC_6HF_4)(O_2)[b]	1.990	4	2.369	1
Fe^{+2}P-450-CAM + O_2	2.00	7.8	2.37	1.3
Fe^{+2}CPO + O_2	2.01	7.4	2.37	1.4
FeOEP(PrSH)(CO)	2.01	5.1	2.41	0.8
FeOEP(THT)(CO)	2.00	5.2	2.41	0.4
FeTpivPP(THT)(O_2)[b]	1.99-2.00	4	2.49	1
FeTPP(THT)$_2$[b]	1.996	4	2.336	2
FeTPP(C5Im)(THT)[b]	1.99	4	2.31	1
FeOEP(MeSSMe)(CO)	2.03	5.9	2.40	0.7

a). See reference 6 for abbreviations and references on complexes. b). Based on crystal structure; all others are from EXAFS analysis.

axial ligation in heme proteins and are structurally characterized for the first time herein. The structural properties of both complexes are similar to those of FeOEP(PrSH)(CO) and distinct from those of $FeOEP(SPr)(CO)^-$.

The ferrous-CO complexes with thiol, thioether and disulfide sulfur donor atoms, respectively models for cysteine, methionine, and cystine axial ligation, have been structurally characterized. In the CO-ligated series, the $Fe-S_{ax}$ bond length has been found to vary with the nature of the sulfur donor. Two distinct categories of $Fe-S_{ax}$ distances have been observed that correlate with thiolate and non-thiolate (thiol, thioether, disulfide) ligation (~2.33 and ~2.41 Å, respectively). Based on this study, it should be possible to discriminate between thiolate and non-thiolate axial ligation in sulfur donor-containing ferrous-CO heme proteins using $Fe-S_{ax}$ bond distances.

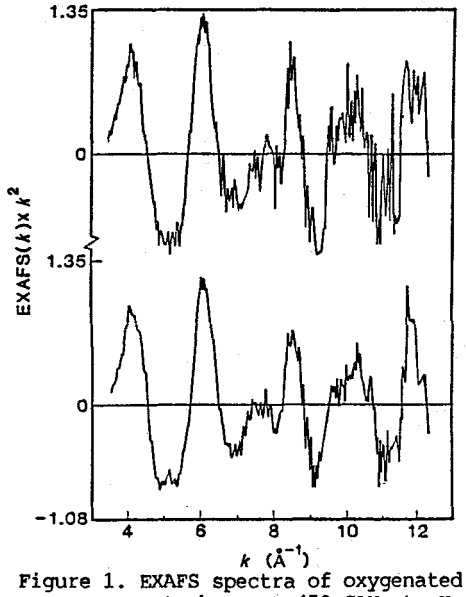

Figure 1. EXAFS spectra of oxygenated cytochrome P-450-CAM at pH 7.4 (top) and oxygenated chloroperoxidase at pH 6.0 (bottom) obtained at ~80 C.

The EXAFS spectra of oxy-P-450 and oxy-chloroperoxidase (Fig. 1) are identical to within the noise level of the data. This provides qualitative support for the proposal [4] that the two complexes have identical heme iron coordination spheres and that differences in their UV-visible absorption and magnetic circular dichroism spectra are due to differences in heme environments [3]. The bond distances and numbers of atoms for $Fe-N_p$(porphyrin), $Fe-S_{ax}$ obtained by curve-fitting analysis of Fourier-filtered first shell data for the two oxygenated enzymes are included in Table 1. The numbers of ligated atoms obtained from curve-fitting analysis are larger than the expected values (i.e., 4 for $Fe-N_p$). 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 [10], $Fe-N_p$ bond distances of 2.00 Å are observed for the dioxygen adducts of both proteins. The contribution of the $Fe-S_{ax}$ backscattering shell is significant as can be seen in Figure 2. The $Fe-S_{ax}$ bond lengths of 2.37 Å found for both oxygenated enzymes provide quantitative evidence that their coordination structures are very similar. The Fe(II)-S bond length of 2.37 Å is identical to that reported by Weiss and co-workers for a thiolate/O_2 ferrous porphyrin model $FeTpivPP(SC_6HF_4)(O_2)^-$ [10] (Table 1) and is longer than that in the corresponding CO-adduct of P-450 (2.32 Å) [11]. Lengthening of the $Fe-S_{ax}$ 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 and O_2, respectively) [12]. Unfortunately, the report of two different $Fe-S_{ax}$ bond lengths (2.352 [13] and 2.388 Å [14]) for $FeTTP(SEt)(CO)^-$ and the use of the highly electron-withdrawing 2,3,5,6-tetra-fluorobenzenethiolate as the axial ligand of the O_2-bound model complex makes it difficult to evaluate the CO/O_2 trans effect in the case of the previously characterized crystalline thiolate model complexes. Nonetheless, the trends observed here for Fe(II)-S bond distances for CO-ligated complexes with the aforementioned Fe(II)-S bond distances with thiolate and thioether trans to O_2 allows the sulfur donor in oxy-P-450 and oxy-CPO to be idenfied as thiolate (cysteinate).

In summary, a systematic study of ferrous-CO heme complexes with sulfur donor ligands trans to CO has reveals a ~0.1 Å lenthening in the Fe-S

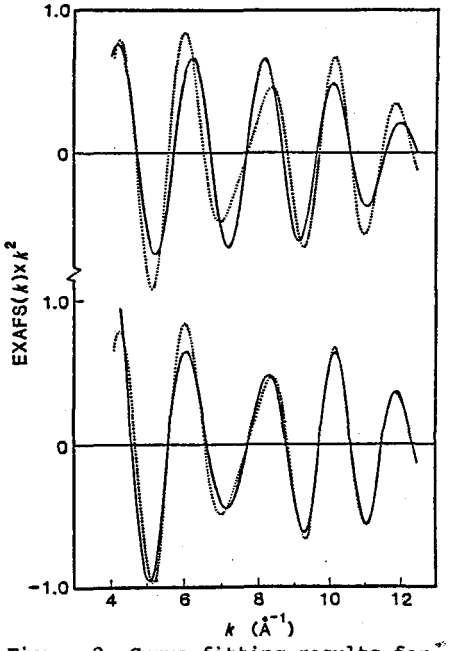

Figure 2. Curve-fitting results for oxy-P-450-CAM. The least square fit (solid line) to the first shell filtered data (dot line) without (top) and with (bottom) inclusion of the Fe-S(axial) shell.

bond distance for thiol, thioether and disulfide relative to thiolate. These results substantially expand the database of Fe-S_{ax} bond lengths in ferrous porphyrin complexes. EXAFS analysis of oxy-P-450 and oxy-CPO has revealed a sulfur donor ligand at 2.37 Å in each case. Our model compounds results and available Fe-S bond distances in ferrous model heme complexes are used to argue that the iron-bound sulfur atom in CO- and O_2-bound P-450 and O_2-bound CPO is a thiolate. Oxy-P-450 is the last stable intermediate in the P-450 reaction cycle and the P-450 state about which the least has been known; its structural characterization and the parallel results with oxy-CPO represent significant breakthroughs in our understanding of these enzymes.

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