Characterization of Active Center of a Non-Heme Chloroperoxidase by X-Ray Absorption Fine Structure

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XAFS studies on this non-heme chloroperoxidase reveal that its Fe core is in a saturated six-coordinate, octahedral environment with four planer N atoms and two axial S atoms. The active site is very similar to Fe porphyrine center in P450 oxidase, but the coordinated N atoms come from amino acids of polypeptides or other molecules, but not porphyrin.

The chloroperoxidase (CPO) catalyzes the formation of carbon halogen bonds between I^-, Br^-, Cl^-, and halogen acceptors such as β-keto acids, AH (reaction 1). This two-electron oxidative process occurs with concomitant reduction of hydrogen peroxide to water.

$$AH + X^- + H^+ + H_2O_2 \xrightarrow{\text{chloroperoxidase}} AX + 2H_2O \quad (1)$$

Most of CPOs have been found to contain heme-iron active sites, very similar to the ferric form of cytochrome P450 oxidase.[1,2] They show a UV-Vis absorbance feature, named as Soret band. The catalytic mechanism and active site of these CPOs have been extensively studied by using EPR,[3] optical absorption,[4] MCD,[5] and XAFS[6] spectral methods. Likewise, few of CPOs have been thought to have non-heme metal active sites which should be inactive in Soret band.[7] However, the details are still remained unclear.

A CPO has been purified from the strain - LC8707 fumago with specific activity of 1120 μ/mg and molecular weight of 58 kDa.[8] The protein does not show Soret band in optical absorption, suggesting that it may not contain non-heme iron active site. In this communication, the further characterization of the active site of the CPO by means of XAFS spectroscopy is reported.

CPO was purified as described previously.[8] It was dialyzed against 0.01 mole/L phosphate buffer (pH=7.0) for 8 h, and then prepared to solid protein sample by using dry-cooling method. XAFS data were recorded at room temperature in a fluorescence mode using the BL-7C facilities at Photon Factory. The energy scales of spectra were calibrated using an Fe foil with the first inflection point defined at 7111.2 eV, EXAFS data were analyzed as described previously.[9]

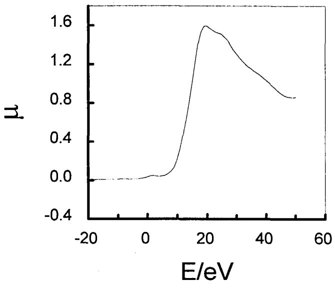


Figure 1 Iron K-edge XANES spectrum of the non-heme chloroperoxidase.

The XANES spectrum of this CPO has been shown in Figure 1. A very weak pre-edge absorption is observed. Previous XANES studies and molecular calculations on ferric model compounds have demonstrated that the intensity of the pre-edge (1s→3d transition) relative to the edge height jump can be correlated with the metal coordination environment.[10] In general, the peak intensity decreases as the iron coordination environment changes from tetrahedral to octahedral, $I_{\text{tetrahedral}} > I_{\text{5-coordinate}} > I_{\text{octahedral}}$. A very weak pre-edge absorption in XANES spectrum of this protein indicates that this iron core may contain a 6-coordinate iron site.

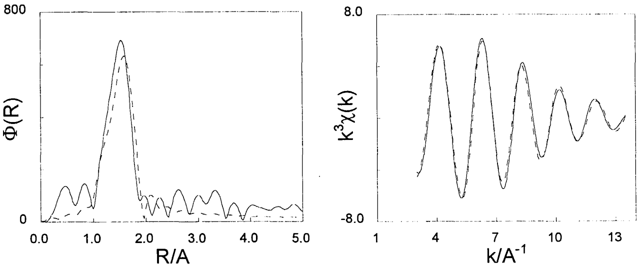


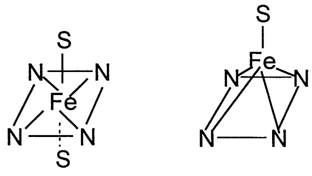
Figure 2 The best (dashed lines) to the filtered first shell EXAFS in R space (left, 0.99- 2.24Å) and in k space (right, 2.4-13.5Å^{-1} with the weight of k^3).

Figure 2 shows that the iron core has rather simple near neighboring environment. Besides the strongest peak similar to the Fe-N_p shell in FeTPP, other main contributions appeared in the Fourier transform of FeTPP are not found in the transform of this CPO. It seems that the iron core of the protein does not contain porphyrin ring. The α, β, γ-tetraphenylporphinatoiron (II) (FeTPP) serves as a useful benchmark for the understanding of the Fourier transform of iron porphyrin EXAFS because of its high symmetry and lack of axial ligands. The details reported by Hodgson and his co-workers[6] have indicated that the first and the second strongest contributions in the transform of the FeTPP EXAFS arise from the four Fe-N_p and the eight Fe-C_α interactions. The four Fe-C_γ interactions contribute only minor to the EXAFS, which is often not resolvable from the Fe-C_α peak. The Further contribution due to eight Fe-C_β interactions is also observed. These features have been also found in those proteins containing heme-iron center.[11] However, this CPO does not exhibit the spectral features of FeTPP and heme-iron proteins at all, as shown in Figure 2. It provides further evidence that the CPO has non-heme iron center. This result is consistent with that obtained from UV-Vis spectroscopic studies on this protein.

Table 1 The EXAFS curve results of the CPO (CPO)

Protein or compd.	Fe-N		Fe-Cα		Fe-S		Ref.
	R (Å)	Number	R(Å)	Number	R(Å)	Number	
Fe(TPP)	1.98	4.4	3.04	7.7	--	--	6
CPO	1.97	4.5	--	--	2.15	2.1	this work
heme CPO	2.04	4.2	3.09	5.7	2.30	0.9	6
cyto P_{450}	2.00	4.0	3.06	5	2.20	1.4	6

The curve-fitting procedures are performed according to the method described previously,[9] and the results are shown in Table 1and Figure 2 (dashed lines). The curve fitting analysis to the EXAFS reveals that the coordination number of the nearest N atoms surrounding iron core is about 4 at a distances of 1.97 Å (Debye-Waller factor: 0.0005 Å). The coordination number of S atom is about 2 at a distance of 2.14 Å (DW: 0.0012 Å). The iron core of the non-heme CPO has four N atoms and two S atoms in the nearest neighboring shell on average. The coordinated atoms, N and S may come from amino acids of polypeptides or other molecules, but not porphyrine molecule. It is noteworthy that the coordination environment of the iron core in this CPO, in which four N atoms may build a plane like that in porphyrine, is very similar to the heme CPO and cytochrome P450 oxidase as compared in Table 1. EXAFS studies well explain why the protein has very similar biological functions but shows very different UV-Vis spectroscopic properties with those of heme CPO.



A B

Figure 3 The proposed structure of the active site of the non-heme chloroperoxidase.
A: the non-heme CPO. B: the heme CPO (ref. 6)

The slightly difference on iron cores between two kinds of CPO could be found, that is, 6-coordination iron core in non-heme CPO while 5-coordination iron core in heme CPO. The EXAFS studies on heme CPO reveal that the iron core is high-spin (Fe out of plane) in the porphyrin center with an axial cysteine thiolate bound to it.[6] In comparison with the coordination environment of iron center in P450 oxidase shown in Table 1, the iron center in the CPO reported here has surprised similarities on coordination number and bond length of S and N shells, suggesting that it contains a low-spin (Fe in the plane of four coordinated N atoms) iron center with two axial S atoms bound to the iron, as shown in Figure 3. To our knowledge, the active center in CPO has not been reported before.

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