Cobalt Porphyrin Heme Oxygenase Complex. EPR Evidences for the Distal Heme Pocket Hydrogen Bonding

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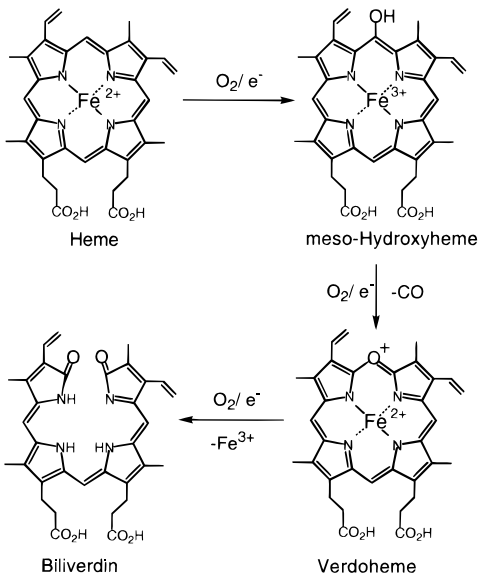
Heme oxygenase (HO) is the central enzyme of the microsomal heme degradation pathway.[1,2] The enzyme binds one equivalent of hemin to form the ferric heme−HO complex.[2] The hemin iron in the complex is reduced to its ferrous form by the first electron donated by NADPH-cytochrome P-450 reductase. The molecular oxygen then binds to form a metastable oxy-form.[3] Electron donation to the oxy form initiates the three consecutive cycles of mono-oxygenation through the α-*meso*-hydroxyheme and verdohemeIXα intermediates during which CO, iron, and biliverdinIXα are produced (Scheme 1).[4,5] The first mono-oxygenation cycle of the HO catalysis, where heme is converted to α-*meso*-hydroxyheme, has been proposed to proceed by reduction of the pseudo-stable O_2-bound complex to a hydroperoxide active intermediate.[6] This is different from the ferryl-oxo active form of cytochrome P-450 enzymes.[5] The formation of the ferric hydroperoxide active intermediate is consistent with the neutral imidazole axial ligation of the heme complex.[6−8] One of the axially coordinated oxygen atoms, presumably the terminal oxygen, then attacks the α-meso carbon of the porphyrin ring and hydroxylates it. The structure of the Fe−O_2 unit, a direct precursor of the oxygen-activated form, is expected to reflect the distal interactions present in the activated complex.

On the basis of resonance Raman spectra of the O_2-bound form of the heme−HO complex, a highly bent Fe−O−O geometry has been proposed.[9] The terminal oxygen atom could be placed in a van der Waals contact with the α-meso carbon of the porphyrin ring, thereby facilitating the regiospecific hydroxylation. While ^{1}H NMR studies of heme−HO complex and model studies of heme degradation reaction have suggested the importance of hydrogen-bond interaction between the bound ligand and the distal heme pocket in the HO heme degradation reactions,[10] the interaction of bound oxygen with the distal amino acid residue has not been identified. In this study, we have carried out EPR measurements of the cobalt(II) porphyrin HO complex. We report that the bound-O_2 forms hydrogen-bond interactions with distal amino acid residues.

Scheme 1. Reaction Intermediates in the Heme Oxygenase Catalyzed Oxidation of Heme to BiliverdinIXα



Cobalt(II) porphyrin HO complex (cobalt−HO) was prepared by combining the cobalt(II) porphyrin with HO under anaerobic condition and purified with an anaerobic Sephadex G-25 column equilibrated with 0.1 M phosphate buffer, pH 7.0.[11] Optical absorption spectra of the oxy and deoxy forms of cobalt−HO are similar to those of the cobalt(II) porphyrin-substituted myoglobin (cobalt−Mb).[12] Figure 1 shows the EPR spectra of the deoxy forms of cobalt−HO and cobalt−Mb. Deoxy cobalt−HO exhibits an EPR spectrum of axial symmetry which is typical of a five-coordinate cobalt(II) complex with a nitrogenous base axial ligand. The $g_{||}$ signal of deoxy cobalt−HO exhibits an octaplet hyperfine structure due to the hyperfine interaction with the ^{59}Co ($I = {}^7/_2$, 100%) nucleus. Each of the hyperfine lines is further split into triplet by hyperfine interaction with a nitrogen atom (^{14}N; $I = 1$, 99.6%) of the proximal axial ligand. The g values and hyperfine coupling constants of deoxy cobalt−HO are estimated as $g_\perp = 2.310$, $g_{||} = 2.027$, $A_{||}(^{59}\text{Co}) = 8.00$ mT, and $A_{||}(^{14}\text{N}) = 1.80$ mT using a computer EPR simulation (Figure 1c). The EPR parameters of deoxy cobalt−HO are close to those of deoxy cobalt−Mb ($g_\perp = 2.327$, $g_{||} = 2.037$, $A_{||}(^{59}\text{Co}) = 7.70$ mT, and $A_{||}(^{15}\text{N}) = 1.67$ mT), which has been characterized as a five-coordinate Co(II) state by X-ray crystallography.[13] The axial N-base coordination in deoxy cobalt−HO is consistent with the His25 proximal axial ligand in the heme−HO complex determined in our previous studies.[7]

Figure 2 shows the EPR spectra of oxy cobalt−HO and oxy cobalt−Mb. Oxy cobalt−HO exhibits a free radical type spectrum centered around $g = 2$ as oxy cobalt−Mb,[12,14] indicating that O_2

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(8) Our spectroscopic studies, as well as others, have shown that the axial ligand of the heme-HO complex is a neutral histidine, in contrast to the cysteine anion present in P-450s.[5] This implies that the enzyme has abandoned the vectorial polar environment across the heme which stabilizes the high oxidation state of the ferryl-oxo (Fe^{4+}═O) intermediate, the oxygen-activated form of cytochrome P-450s.
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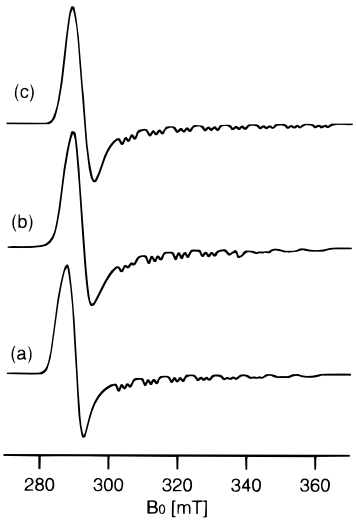

Figure 1. EPR spectra (20 K) of deoxy cobalt−HO and deoxy cobalt−Mb in 0.1 M phosphate buffer, pH 7.0. Conditions: frequency, 9.453 GHz; incident microwave power, 0.10 mW; modulation amplitude at 100 kHz, 0.51 mT; receiver gain, 5.00×10^4. (a) Deoxy cobalt−Mb. (b) Deoxy cobalt−HO. (c) Computer simulation of (b).

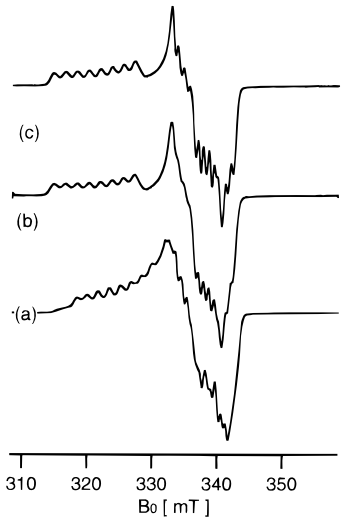

Figure 2. EPR spectra (20 K) of oxy cobalt−HO and oxy cobalt−Mb in 0.1 M phosphate buffer, pH 7.0. Conditions: frequency, 9.453 GHz; incident microwave power, 0.10 mW; modulation amplitude at 100 kHz, 0.20 mT; receiver gain, 2.50×10^4. (a) Oxy cobalt−Mb. (b) Oxy−cobalt−HO. (c) Oxy cobalt−HO in deuterated buffer.

binds to Co(II) in a manner similar to that in the oxy cobalt−Mb system.[13] The spectral parameters of oxy cobalt−HO was estimated by computer simulation as $g_1 = 2.104$, $g_2 = 2.007$, $g_3 = 1.990$; $A_1 = 1.76$ mT, $A_2 = 0.98$ mT, $A_3 = 0.83$ mT. The oxy cobalt−Mb spectrum has been shown to be composed of at least two species, named type I and II, which are distinct in the distribution of unpaired spin over the dioxygen and in the Co−O_2 bonding geometry.[14] The EPR parameters for type I are $g_1 = 2.08$, $g_2 = 2.03$, $g_3 = 1.98$, $A_1 = 0.72$ mT, $A_2 = 2.32$ mT, and $A_3 = 0.62$ mT; and for type II, $g_1 = 2.085$, $g_2 = 2.008$, $g_3 = 1.983$, $A_1 = 1.73$ mT, $A_2 = 0.82$ mT, and $A_3 = 0.77$ mT.[15] Although the EPR parameters of cobalt−HO are closer to those of type II than type I of oxy cobalt−Mb, the EPR spectrum of oxy cobalt−HO is different from that of oxy cobalt−Mb in the following two aspects. First, the *g*-anisotropy of the cobalt−HO spectrum is different from that of cobalt−Mb. The $g_{\|}$ component, g_1, of the cobalt−HO spectrum is more anisotropic than that of oxy cobalt−Mb, while the $g_\perp$ components, g_2 and g_3, are slightly less anisotropic. This is an indication of the different Co−O−O geometry between cobalt−HO and cobalt−Mb and is consistent with the resonance Raman results of the iron counterparts.[9] Second, the spectrum of oxy cobalt−HO consists of a single paramagnetic species as opposed to the presence of at least two species in oxy cobalt−Mb spectrum.[14] The bound dioxygen in oxy cobalt−HO is confined to a well-defined single geometry due to the strong distal pocket interactions as proposed in the resonance Raman studies on the oxy heme−HO complex.[9]

To explore possible hydrogen-bonding interactions between the bound oxygen and amino acid residues in the distal pocket, we have measured the EPR spectrum of oxy cobalt−HO in deuterated phosphate buffer (Figure 2c). Measurement in D_2O significantly sharpens the hyperfine structure of the oxy cobalt−HO spectrum. Similar spectral changes have been observed for oxy cobalt−Mb, which is interpreted as evidence for the hydrogen-bond formation between the bound dioxygen with the distal histidyl proton by the EPR, ESEEM, and ENDOR experiments.[11,16] It is likely that a hydrogen-bond interaction is also present between the bound dioxygen and an amino acid residue in the distal pocket of HO. While the hydrogen bond between the bound oxygen and the distal histidine in Mb is known to stabilize its oxy form,[12,13] the oxy form of HO autooxidizes much faster than oxy Mb.[3] The consequences of the hydrogen bonding are different between HO and Mb. We believe that the hydrogen-bond interaction in HO plays significant roles in the α-hydroxyheme formation (Scheme 1). The hydrogen-bond interaction can orient the bound oxygen to a position which is favorable to regio-specifically oxidize the α-meso carbon of the porphyrin ring.[9] The hydrogen bond interaction is also favorable to the HO catalytic reaction, because the interaction of the bound dioxygen with a proton decreases the reduction potential of oxy HO to form a putative ferric hydroperoxide active species. In fact, when oxy cobalt−HO is reduced by sodium ascorbate or *p*-hydroquinone, bound oxygen is reduced to hydrogen peroxide via the cobalt(III) hydroperoxide complex.[17,18] The proton donation via hydrogen-bond interactions, as proposed for cytochrome P-450,[5,19] converts oxy HO to the ferric hydroperoxide HO complex, which then leads to the formation of the α-*meso*-hydroxyheme HO complex. As the reactivity of cytochrome P-450 is modulated by a hydrogen bond to the bound oxygen,[19] the heme oxygenase activity may be controlled by the hydrogen-bond interaction revealed in this study.

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(18) The reactivity of oxy cobalt−HO with the reductants was identical to that of the oxy-form of cobalt-substituted horseradish peroxidase.[17] Addition of the physiological electron donor (NADPH and NADPH cytochrome P-450 reductase) to oxy cobalt−HO did not form biliverdin. However, addition of excess hydroquinone converted oxy cobalt−HO to its Co(III) form without porphyrin degradation. The reaction can be interpreted that the reduction of oxy cobalt−HO produces the Co(III) hydroperoxide complex, which then converts to Co(III) porphyrin−HO and hydrogen peroxide. No absorption spectral change was observed when hydrogen peroxide was added to the Co(III) porphyrin−HO complex. On the other hand, in accordance with the previous report by Hoffman et al., when hydroquinone was added to oxy cobalt−Mb under aerobic condition, a yellow-green compound formed irreversibly, having a characteristic absorption band at 623 nm in addition to the normal cobalt porphyrin α-, *β*-, and *γ*-bands.[17.]

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