PS1.41 — TU

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SPECTROSCOPIC PROPERTIES OF CHLOROPEROXIDASE COMPOUNDS II AND III — POSSIBLE STRUCTURAL MODELS FOR ANALOGOUS CYTOCHROME P-450 DERIVATIVES

Previous spectroscopic studies of analogous derivatives of chloroperoxidase (CPO) and cytochrome P-450 (P-450) have demonstrated that close similarities exist in their UV-visible absorption [1], magnetic circular dichroism (MCD) [2], EPR [3], Mössbauer [4], and extended X-ray absorption fine structure [5] properties. In particular, both enzymes exhibit unique hyperporphyrin («split Soret») spectra in their ferrous-CO [1] and ferric-thiolate [6] adducts, strongly suggesting the coordination of an endogenous thiolate ligand in CPO, as has well been established for P-450 [7]. These extensive parallels between the coordination properties of CPO and P-450 have motivated us to generate other CPO derivatives as potential analogs for catalytic intermediates of P-450, especially its oxygen complexes.

We report herein the generation of a unique, stable CPO-dioxygen adduct ($CPO \cdot O_2$, CPO Compound III) and of CPO Compound II(CPO-II), and their characterization with UV-visible absorption and MCD spectroscopy. The CPO dioxygen adduct is formed by bubbling O_2 into a dithionite-reduced enzyme solution at $-30°C$ in cryogenic mixed solvents. CPO-II is generated at ambient temperature ($\sim 4°C$) by adding peroxides and ascorbic acid to ferric CPO (1-2 μM) in a molar ratio of 100:2000:1. The UV-visible absorption and MCD spectra of $CPO \cdot O_2$ and CPO-II are displayed in figs. 1 and 2, respectively.

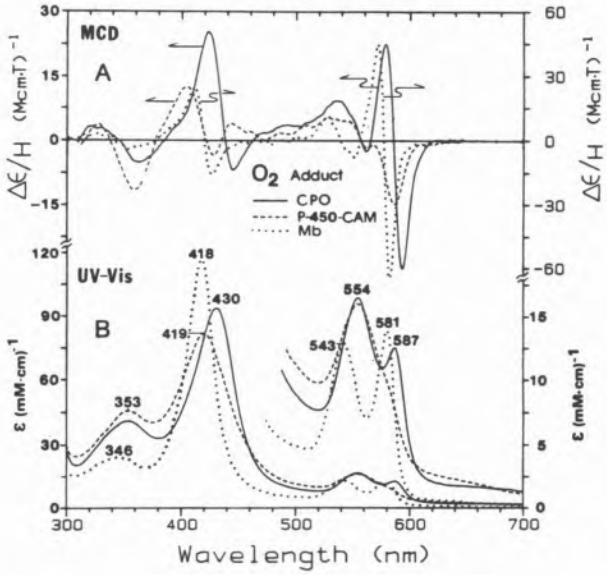

Fig. 1
UV-visible absorption (bottom) and MCD (top) spectra of oxygenated CPO (———), P-450-CAM (------) and Mb (·····). The spectra were obtained in 65% (v/v) ethylene glycol/0.035 M potassium phosphate buffer (pH 6.0, 7.4 and 7.0 before mixing, respectively) at −30°C with 30-40 μM protein concentrations. The P-450-CAM sample contained 100 mM KCl and 4 mM d-camphor

The absorption spectrum of $CPO \cdot O_2$ at $-30°C$ (Fig. 1B) [$\lambda_{nm}(\epsilon_{mM})$:354(41), 430(94), 554(16.5), 587(12.5)] has two noticeable distinctions from that of $P\text{-}450 \cdot O_2$: (a) the Soret peak of $CPO \cdot O_2$ is red-shifted about 10 nm from that of $P\text{-}450 \cdot O_2$, and (b) a distinct α-peak is seen at 587 nm in the CPO case that is not observed for $P\text{-}450 \cdot O_2$. However, the overall spectral features of $CPO \cdot O_2$ are more similar to those of $P\text{-}450 \cdot O_2$ [$\lambda_{nm}(\epsilon_{mM})$:353(46), 419(82), 554(16)] than of oxygenated myoglobin. Oxygenated CPO is EPR silent at 77 K. The bound O_2 in oxygenated CPO can be replaced by CO; upon bubbling CO into the $CPO \cdot O_2$ solution at $-30°C$, the diagnostic hyperporphyrin spectrum of ferrous-CO CPO ($\lambda_{max,nm}$:362, 445, 549) is generated. $CPO \cdot O_2$ undergoes autoxidation to form native ferric CPO without any detectable intermediates with a half life comparable to that of $P\text{-}450 \cdot O_2$. In fig. 2,

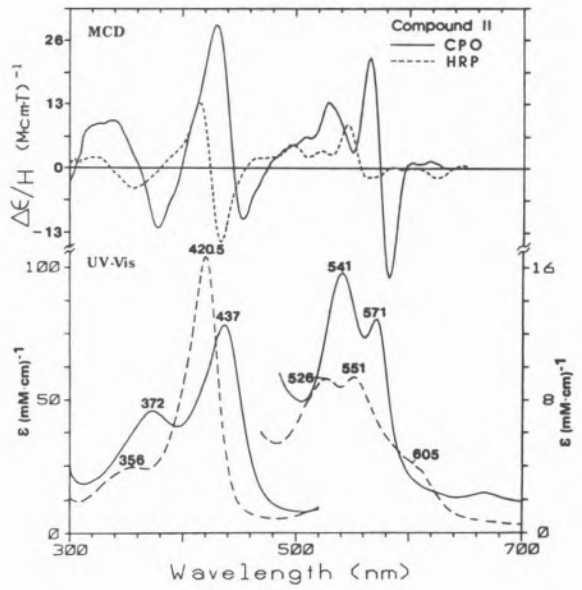

Fig. 2

UV-visible absorption (bottom) and MCD (top) spectra of Compound II derivatives of CPO (———) and HRP (------). The measurements for CPO (1.5-2 μM) were made in 0.1 M potassium phosphate buffer, pH 6.0, with peroxides (~ 0.15 mM) and ascorbic acid (~ 3 mM) and those for HRP (10-15 μM) in 0.005 M sodium carbonate buffer, pH 10.5, with equivalent amounts of EtOOH and ascorbic acid, at ~ 4°C. The MCD spectrum of CPO Compound II is the average of 3 measurements

UV-visible absorption (bottom) and MCD (top) spectra of horseradish peroxidase Compound II (HRP-II) are overplotted with those of CPO-II. Considerable differences are seen between the spectra of HRP-II and CPO-II, presumably because the endogenous axial ligands are not the same: histidine imidazole for HRP and thiolate, most likely, for CPO.

In conclusion, the dioxygen adducts of CPO and P-450 are clearly distinguishable from that of Mb, a histidine-ligated heme protein, in their UV-visible absorption and MCD spectral properties [8]. Considerable spectral differences are also seen between the Compound II states of CPO and HRP. The small, but nonetheless significant, spectroscopic dissimilarities between the dioxygen adducts of CPO and P-450 (Fig. 1A, B) are somewhat surprising, since the analogous ferric and ferrous ligand adducts of CPO and P-450 show great similarities [1,6,9]. The reason for these differences are not clear at the present.

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PS1.42 — TH

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ANION BINDING TO A CYTOCHROME *c'*

The cytochromes *c'* are mono- and diheme proteins reported to comprise the largest and most widespread class of bacterial cytochromes known [1]. These proteins are similar to the low-spin