PS2.4 — MO

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MCD STUDIES ON THE HEME AND TRYPTOPHAN COMPONENTS OF CYTOCHROME *c* PEROXIDASE

We have measured the magnetic circular dichroism of cytochrome *c* peroxidase (CCP) and some of its derivatives from 250-350 nm. Comparison of the changes observed on conversion to the catalytic intermediate (CCP-I) with spectra obtained from horseradish peroxidase and its derivatives and model compounds of protoheme leads us to the conclusion that the observed changes in the MCD spectra reflect conversion of the heme to the ferryl state. No evidence was found for modification of tryptophan in CCP-I.

PS2.5 — TU

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MAGNETIC CIRCULAR DICHROISM STUDIES OF CYTOCHROME *c* PEROXIDASE AND ITS LIGAND COMPLEXES

INTRODUCTION

Yeast cytochrome *c* peroxidase (CCP) is a soluble heme protein, located in the mitochondrial intermembrane space, that catalyzes the two-electron reduction of hydroperoxides by ferrocytochrome *c* in the following reaction:

$$2\ \text{cyt}\ c(Fe^{2+}) + ROOH + 2H^{+} \rightarrow 2\ \text{cyt}\ c(Fe^{3+}) + ROH + H_2O$$

CCP contains a noncovalently bound protoheme IX prosthetic group and has a known amino acid sequence. The crystal structure of CCP has recently been published [1]. Despite the above information, questions still remain about the relationship between the physical structure of CCP and its catalytic properties. We have used magnetic circular dichroism (MCD) spectroscopy to probe the electronic, and therefore indirectly the physical, structure of native ferric and ferrous CCP and its complexes with CN^-, N_3^-, F^-, CO, NO and of CCP-Compound I. In order to provide a basis for comparison, the MCD data on CCP, reported here for the first time, have been compared to analogous data from other imidazole-ligated heme proteins such as myoglobin (Mb) and horseradish peroxidase (HRP).

EXPERIMENTAL PROCEDURES

CCP was purified from baker's yeast [2] to $A_{408}/A_{280} = 1.28$. The enzyme concentration was determined from $E^{mM}_{408} = 93$ [3]. Absorption and MCD spectra were obtained as previously described [4]. Samples were examined in 0.1 M potassium phosphate buffer, pH 7.0, at 4°C except where noted.

RESULTS

The MCD spectra of native ferric and alkaline ferric CCP differ from those of the analogous forms of Mb and HRP (data not shown, [5]), presumably as a result of different ligands at the sixth coordination site. Therefore, we have looked at complexes of native ferric CCP, HRP and Mb with known sixth ligands such as F^-, CN^-, N_3^-. The MCD spectrum of the ferric CCP cyanide complex is very similar to the spectra of analogous Mb and HRP ligand adducts [5]. As shown in Fig. 1, the MCD spectra of the azide complexes of CCP, HRP and Mb are very similar in the Soret region, but differ in the visible region, where the spectrum of Mb-N_3 is somewhat different. Ferric CCP-F also has an MCD spectrum that is much more similar to HRP-F than to Mb-F. The native ferrous forms of all three heme proteins are high spin with imidazole at the fifth coordination site. Once again, the MCD spectra of all three proteins are quite similar in the Soret region but, in the visible region, the spectrum of Mb differs noticeably from those of CCP and HRP (Fig. 1). On the other hand, the low-spin ferrous complexes of all three heme proteins with CO and NO display very similar MCD spectra [5]. Lastly CCP-Compound I exhibits a MCD spectrum that is reasonably similar to HRP--Compound II and differs significantly from that of HRP-Compound I (Fig. 2).

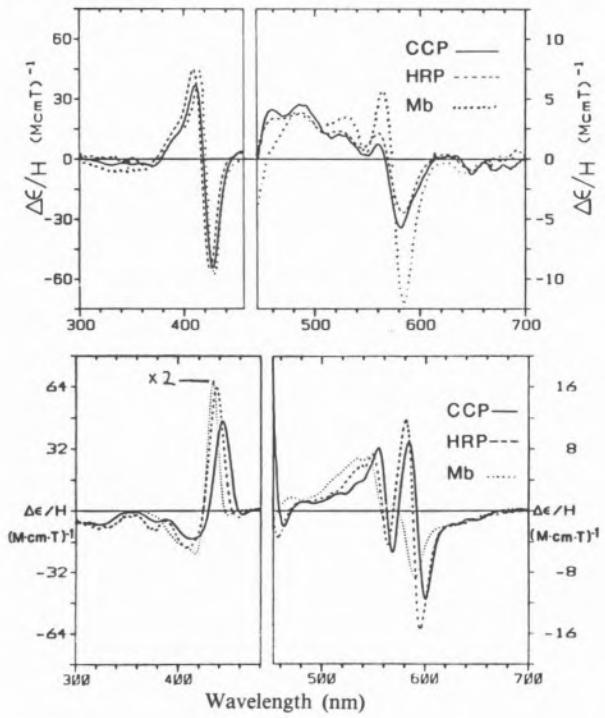

Fig. 1

Top: MCD spectra of the azide complexes of ferric CCP (——), HRP (-----) at pH 4.0 and Mb (·····). Enzyme concentrations were 20-35 μM. Bottom: MCD spectra of high spin ferrous CCP (——), HRP (-----) and Mb (·····). Enzyme concentrations were 50-65 μM

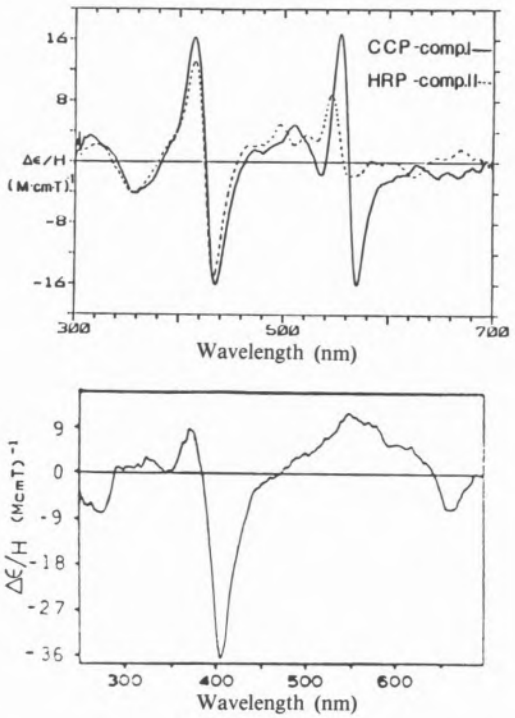

Fig. 2

Top: MCD spectra of 10.3 μM CCP-Compound I (——) and of 13.6 μM HRP-Compound II (-----) in 5 mM potassium carbonate, pH 10.5, with equivalent amounts of EtOOH and ascorbate. Bottom: MCD spectrum of HRP-Compound I at 0°C (taken from [7])

CONCLUSIONS

a) Despite similar ligand environments, some differences do exist among the electronic structures of the three imidazole coordinated heme proteins.

When differences exist, CCP and HRP are similar and Mb is different.

b) CCP-Compound I has a similar electronic structure to HRP-Compound II, an Fe(IV) heme iron complex, and differs substantially from HRP-Compound I, which is thought to have an Fe(IV) iron coupled to a porphyrin π cation radical [6]. This result is consistent with the assignment of CCP-Compound I as a Fe(IV) heme iron with an adjacent oxidized amino-acid side chain radical [6].

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PS2.6 — TH

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OBSERVATION OF THE $Fe^{IV}=O$ STRUCTURES OF HORSERADISH PEROXIDASE COMPOUND II AND FERRYL MYOGLOBIN BY RESONANCE RAMAN SPECTROSCOPY

The formation of intermediate enzymatic states upon reaction of peroxides with heme proteins such as peroxidases and catalases are well known [1]. These intermediate states are commonly known as compounds I and II, which are respectively two and one oxidation equivalents above the resting state of the enzyme (an Fe^{III} heme). Our recent resonance Raman spectroscopy investigations have been aimed towards an understanding of the heme structure of horseradish peroxidase compound II, as well as the similar compound, which is formed upon reaction of hydrogen peroxide with metmyoglobin, known as "ferryl myoglobin" [2].

We have observed the $Fe^{IV}=O$ stretching vibrations on the heme groups of both horseradish peroxidase compound II [3] and ferryl myoglobin [4], giving direct evidence for an $Fe^{IV}=O$ (oxyferryl) heme structure. In horseradish peroxidase compound II we have identified the $Fe^{IV}=O$ stretch at 779 cm^{-1}. Upon ^{18}O substitution the band is observed to shift to 743 cm^{-1}. Additionally we have observed the $Fe^{IV}=O$ stretching vibration at 797 cm^{-1} in ferryl myoglobin. This is confirmed by a shift to 771 cm^{-1} upon reaction of samples with ^{18}O-labelled H_2O_2 [5]. No change in the $Fe^{IV}=O$ frequencies were observed when horseradish peroxidase compound II or ferryl myoglobin were prepared with $^2H_2O_2$. The frequency