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MAGNETIC CIRCULAR DICHROISM SPECTRA OF MODELS FOR THE REDUCED CYTOCHROME P-450 AND ITS OXYGENATED FORM

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The complex formation of dioxygen with mercaptide-heme was first confirmed by the magnetic circular dichroism spectroscopy. Comparison of the spectra of the model complexes with those for cytochrome P-450 revealed the thiolate coordination in the reduced high spin cytochrome P-450 and its oxygenated form.

Cytochrome P-450's comprise a group of hemoproteins which are involved in binding and activation of molecular oxygen and subsequent mixed-function oxidation of a variety of substrates.[1,2] These enzymes were first distinguished by their unusual absorption spectra in their reduced CO complexes which exhibit Soret absorption spectra near 450 nm in contrast with that near 420 nm for the CO complexes of other hemoproteins such as hemoglobin and myoglobin.[3,4] Previous comparative physical properties of synthetic and native porphyrin complexes with various ligands have provided convincing evidence for cysteinate ligation in cytochrome P-450 in their oxidized low and high,[5-9] and reduced high,[10,11] and low spin CO[12-15] and NO[16] complexes. Whereas, in despite of intensive studies there are still controversies about the nonporphyrin ligand to the central iron in the oxygenated P-450.[17,18] Since the magnetic circular dichroism (MCD) spectra of oxygenated P-450 were different from those for oxymyoglobin, this supplied evidence against the axial histidine ligation to the heme iron in the oxygenated P-450.[19-22] In the present letter we wish to communicate absorption and MCD spectra which have afforded evidence for the thiolate ligation in the oxygenated cytochrome P-450 as well as its reduced high spin state.

The complexes were prepared by the similar methods to those described in the

literature[16] for the reduced CO or NO complex. Dimethylsulfoxide (DMSO) (Wako, Spectrasole grade) deoxygenated by equilibriation with argon, was saturated with sodium methylmercaptide (0.32 mol dm^{-3}) followed by the anaerobic addition of dibenzo-1,4,7,10,13,16-hexaoxacyclooctadeca-2,11-diene (dibenzo-18-crown-6 ether) (0.14 mol dm^{-3}) in order to enhance the dissociation of sodium mercaptide to its anion in the solvent. The reagent grade crown ether (Nippon Soda Co. Ltd.) was purified by 2 times recrystallization from benzene. Prior to adding dioxygen the solution of sodium methyl mercaptide and the crown ether was mixed in a Thunberg optical cell with hemin chloride dissolved in DMSO (5×10^{-5} mol dm^{-3}) under argon. After taking the absorption and MCD spectra, dioxygen was bubbled intermittently into the solution for certain periods (10, 20, and 50 s).

Before adding dioxygen the system exhibited the absorption (bottom) and the MCD (top) spectra shown in Fig. 1 which also includes those for the reduced cytochrome P-450 cited from the reference 20. Both absorption and MCD similarity confirmed the formation of the reduced high spin heme complex with one thiolate anion.

When dioxygen was bubbled into the solution, a new spectral species with the absorption maximum at 436 nm was formed. Thus, a shoulder in the

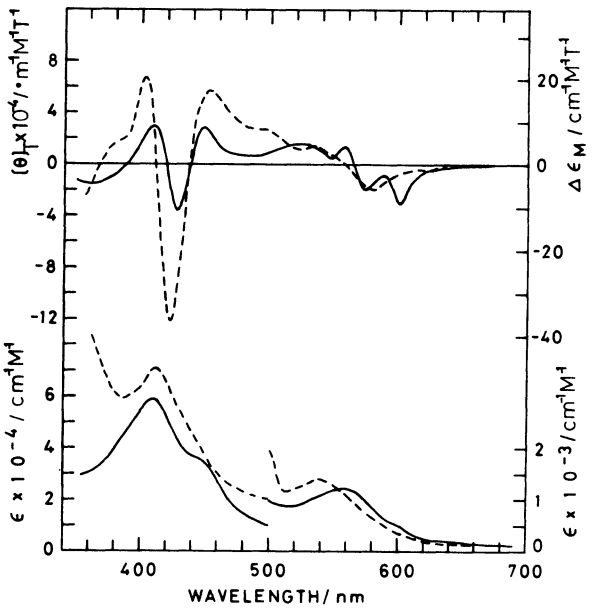

Fig.1. MCD (top) and absorption (bottom) spectra of the reduced thiolate heme (———) and reduced cytochrome P-450 (-----) cited from the reference 20.

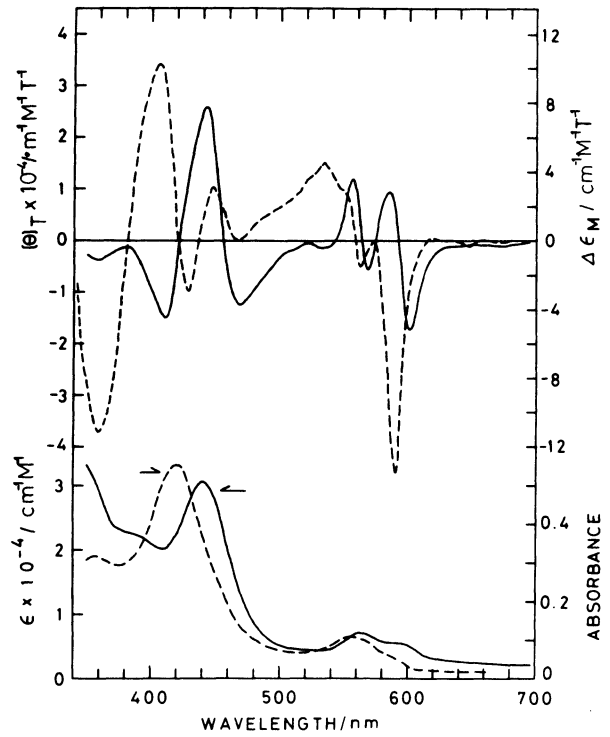

Fig.2. MCD (top) and absorption (bottom) spectra for the reduced thiolate heme after bubbling dioxygen for 50 s (———). The broken lines (-----) demonstrate the MCD (top) and absorption (bottom) spectra for the oxygenated cytochrome P-450 cited from the references 19 and 22.

absorption spectra appeared after 10 s, and it grew to a separate peak after 20 s. Further bubbling for 30 s, the new peak became the main one with a shoulder around 418 nm for some remained original species, after the correction of which the system exhibited the absorption (bottom) and MCD (top) spectra shown in Fig. 2. The new spectral species was most probably identified as an oxygenated heme (iron(II)) complex with a thiolate anion from the following several reasons.

1) Oxidation to hemin (iron(III)) complexes was rather slow in the present system with excess CH_3S^- (a reductant). The system became to μ-oxo-hemin (iron(III)) dimer after loss of CH_3S^- by bubbling dioxygen for 120 s after 5 hours, when precipitate appeared extensively. Further the iron(III) dimercaptide hemin complex should exhibit the absorption peak at ~475 nm. 2) The absorption and MCD spectra demonstrate resemblance to those recently reported[19,22] for the complex claimed as the oxygenated cytochrome P-450 shown by the broken lines in Fig. 2. That is, the MCD spectrum for this new species shows the positive (~442 nm) and negative (~446 nm) MCD bands for the Soret transition, which is characteristic for the iron(II) low spin chromophore. In addition, the visible MCD spectrum affords two apparent Faraday A terms for Q_{0-0} and Q_{0-v} transitions which are also the finger prints as the iron(II) low spin species. 3) The CO complex with the thiolate anion can be formed by bubbling CO gas into the solution with the present species.

It is important to determine the non-porphyrin axial ligands of cytochrome P-450 for understanding its enzymic function. The ligation of thiolate in the oxidized (iron(III)) low and high spin states has been established by synthetic and physico-chemical studies of the model compounds.[5-9] In the reduced (iron(II)) states the ligation of thiolate has been identified in the CO and NO complexes.[12-16] The present MCD spectrum which shows close resemblance to those for the reduced high spin cytochrome P-450 gave further evidence for the thiolate ligation to the heme in the high spin reduced state which has been proposed from the similarity in the absorption spectra.[10,11]

Though the present dioxygen thiolate heme complex in DMSO shows a difference by 18 nm in the Soret band wavelength position from that of oxygenated cytochrome P-450, the complex can be taken as a model compound for the native oxygenated cytochrome P-450. The wavelength shift by 18 nm is attributable at least some part to the nature of the solvent, since the CO thiolate heme complex as the model reduced CO complex of cytochrome P-450 exhibited the absorption maximum at 460 nm in this solvent and it could be shifted by about 10 nm to higher energy (i.e., 450 nm)

reminiscent of carbon monoxy cytochrome P-450 in a 1 : 1 DMSO-ethanol solvent mixture.[16]

In conclusion the present model mercaptide heme complexes established the thiolate coordination in the reduced high spin cytochrome P-450 and suggested the occurence of thiolate ligation in the oxygenated cytochrome P-450.

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