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A Model for the Carbonyl Adduct of Ferrous Cytochrome P_{450}

Sir:

Cytochrome P_{450}[1] is the name given to the active site in a widespread class of hemoproteins which activate molecular oxygen during the hydroxylation of C–H bonds in metabolism, hormone regulation, and drug detoxification. This cytochrome derives its name from the unusual 450-nm Soret band exhibited by its ferrous carbonyl derivative. The position of this 450-nm peak is different from all other known iron(II) porphyrin carbonyl derivatives. Recent spectral studies of model ferric porphyrin complexes clearly indicate that both the resting, low-spin and substrate-bonded high-spin ferric forms of P_{450} have in common an axial *thiolate* ($R\text{-}S^-$) ligand.[2] In the interim, Stern and Peisach[3] reported that the combination of a ferrous heme, CO, excess thiol, and excess KOH in DMSO–EtOH[4] produces a 450-nm absorbance characteristic of ferrous-carbonyl P_{450} along with another species present in ~50% abundance. Their work implies that thiolate is required to produce the characteristic 450 peak.

We have found conditions which afford the 450-nm peak in ~100% abundance. Addition of a benzene solution of $\text{Na}SCH_3$ solubilized by dibenzo-18-crown-6 macrocyclic ether to Fe(TpivPP)[4,5] or Fe(PPIXDEE) followed by exposure of this solution to CO results in a Soret band at 449 nm (Figure 1). This absorbance is quite sensitive to the polarity of the solvent and of the porphyrin. For example, in DMSO the Soret band for both TpivPP and PPIXDEE is found at 462 nm,[6] and the combination Fe(TPP), CO, and $\text{Na}SCH_3$ in benzene exhibits the corresponding band several nanometers lower.[7] The species giving rise to the 449 absorption is extremely sensitive to O_2 which discharges the 449 peak. Undoubtedly because of this air sensitivity at low porphyrin concentrations (5×10^{-6} *M*), a 50–100-fold excess of $\text{Na}SCH_3$ is required to produce the 449 chromophore;[8] whereas at higher porphyrin concentration (10^{-3} *M*) a one- or twofold excess of $\text{Na}SCH_3$ is sufficient to afford >95% of the 449 peak. It is significant that with *mercaptan* rather than *mercaptide* as the axial base the Soret band for the ferrous carbonyl TpivPP or PPIXDEE derivative is found at 422 nm[6] (Table I).

The other characteristic feature of the absorption spectrum of cytochrome P_{450} is the broad featureless band centered at ~550 nm. Unlike other hemoproteins, the α and β bands of the porphyrin ligand are not clearly separated. The extinction coefficients for both the visible and the Soret band compare very well with those of the natural enzyme, at least in the case of Fe(PPIXDEE).[9]

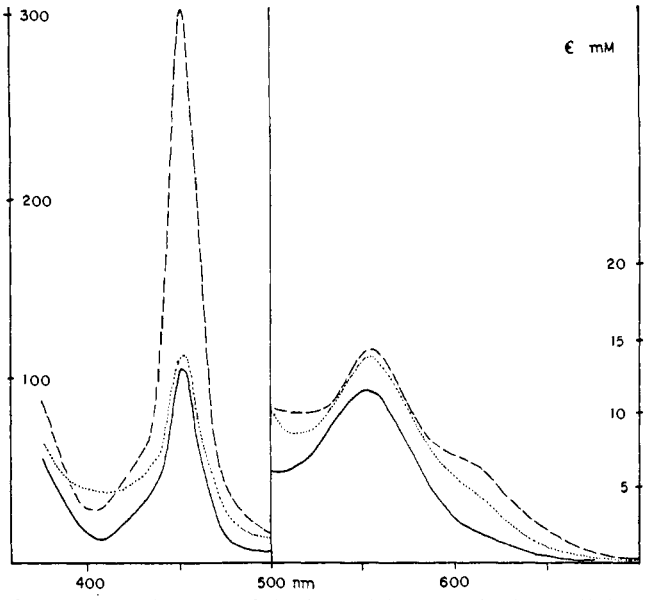

Figure 1. Plots of wavelength (nm) vs. millimolar extinction coefficient for: (—) reduced P_{450} cam + CO at 5° in 50 m*M* potassium phosphate buffer (ref 1), (- - -) Fe(TpivPP) and $\text{Na}SCH_3$ + CO in benzene at 25°, (···) Fe(PPIXDEE) and $\text{Na}SCH_3$ + CO in benzene at 25°.

Table I. Spectral Data for Fe(TpivPP) + B + CO

B	ν_{CO}[a,b]	λ_{max}[a,c]
N-MeIm[f]	1964 (1965)	427[e]
THF[f]	1961[d] (1955)	417[d,e]
THT[f]	1970 (1972)	428
$n\text{-}C_3H_7SH$	1970	422
$\text{Na}SCH_3$	1945 1902[g]	449

[a] Benzene solution; numbers in parentheses refer to spectra obtained in KBr pellets. [b] In cm^{-1}. [c] In nm. [d] THF as solvent. [e] Reference 5. [f] These complexes have been isolated and fully characterized. [g] Spectrum with ^{13}CO.

The prior spectral studies of ferric porphyrins[2] and the present work reproducing the P_{450} ferrous Soret strongly imply that an axial *mercaptide* ligand is present throughout the P_{450} catalytic cycle—encompassing two ferric and two ferrous stages.[1,2a]

The ν_{CO} infrared bands for the carbonyl mercaptide and complexes having other axial bases are listed in Table I. Although the differences are not large, the ν_{CO} value for the complex having an axial mercaptide ligand is the lowest ν_{CO} frequency for a ferrous carbonyl porphyrin of which we are aware. Since ν_{CO} for the enzyme is unknown, comparison cannot be made with the natural system. However, determination of this frequency does set a target for studies with the P_{450} ferrous carbonyl complex. Comparison of ν_{CO} values in Table I supports the intuitively reasonable hypothesis that mercaptan is a better π-acid ligand than mercaptide (which may, in fact, be a π-donor[10]). It seems likely that the mercaptide axial base may have an important electronic effect necessary to activate dioxygen for the subsequent hydroxylation event. This point must remain a speculation until a model dioxygen complex has been prepared and characterized.

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(4) The following abbreviations are used: DMSO = dimethyl sulfoxide, THF = tetrahydrofuran, THT = tetrahydrothiophene, N-MeIm = *N*-methylimidazole, (Na)SCH_3 = sodium mercaptide dibenzo-18-crown-6 complex, TPP = tetraphenylporphyrin dianion, TpivPP = $\alpha,\alpha,\alpha,\alpha$-tetra-(*o*-pivalamidophenyl)porphyrin dianion,[5] PPIXDEE = protoporphyrin IX diethyl ester dianion.
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(6) There are some notable discrepancies between this work and that of Stern and Peisach.[3] For example, in DMSO, they report a peak at 450 nm for the mercaptide species, whereas we find the analogous absorption at 462 nm. For the mercaptan species, they report a peak at 413 nm, whereas we observe a band at 422 nm. It is possible that these differences arise from the fact that they used protoporphyrin IX while we employed the diethyl ester. It is interesting to note that the ratio $A_{450}/A_{555} = 4$ for the previous work, whereas for most cytochrome P_{450}'s this ratio is closer to 10.
(7) Undoubtedly the protein environment plays a role in the exact position of the 450 peak. Recall that the picket fence porphyrin,[5] TpivPP, has four amides on one side of the porphyrin ring and PPIXDEE has two polar ester groups.
(8) Presumably, the air serves to oxidize the ferrous complex to ferric. Excess mercaptide is then necessary to reduce the iron back to the ferrous state. We noted previously[2a] the propensity of ferric mercaptide complexes to reduce spontaneously giving Fe(II) species.
(9) We have already noted[5] that *meso*-tetraarylporphyrins often have different absorption spectra so that it is not unusual that extinction coefficients for FeTpivPP are different from those of the natural system.
(10) This supposition has strong support in the yet unpublished work of Taube and Kuehn comparing stability constants for mercaptide and mercaptan binding to ruthenium(II) and -(III).

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Evidence for the Oxidation of Supersensitizers during Photoelectrochemical Supersensitization at the CdS Electrode

Sir:

Studies on spectral sensitization have recently been carried out by a number of authors using electrochemical procedures,[1-5] principally with the aim of elucidating the mechanism of spectral sensitization. The experimental results reported in the literature can on the whole be interpreted by postulating the electron transfer mechanism (the electron transfer from excited dyes to a semiconductor), which has extensively been discussed in photographic science. Provided that the spectral sensitization at n-type semiconductor electrodes involves the electron transfer mechanism, sensitizing dyes or supersensitizers (reducing agents) should, at least momentarily, be oxidized. However, the detection of oxidized forms of sensitizers or supersensitizers has not thus far been reported.

In the present study we demonstrated the oxidation of supersensitizers in the course of spectral sensitization at the CdS electrode, by means of a rotating ring-disk electrode (RRDE) system, where a CdS single crystal served as the disk electrode and Au as the ring electrode.

The CdS single crystal employed was an n-type semiconductor, with a carrier density of 7.4×10^{16} cm^{-3}. The electrode was a pellet having an average radius of 3.25 mm and a thickness of 1.0 mm. In order to ensure an ohmic contact with a lead wire, indium was vacuum-evaporated onto a small part of the electrode surface. The CdS electrode was mounted in a Teflon rod, together with a gold ring electrode, as illustrated in Figure 1.

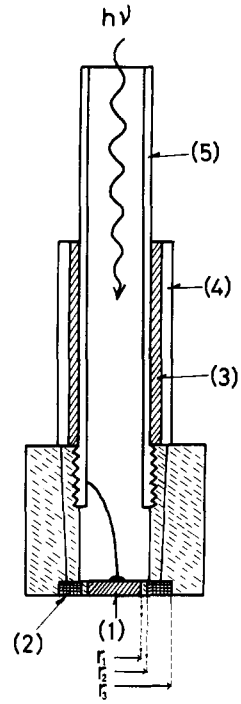

Figure 1. Ring-disk electrode assembly: (1) CdS single crystal (disk electrode); (2) Au (ring electrode); (3) insulator; (4), (5) brass cylinders, $r_1 = 3.25$ mm, $r_2 = 3.75$ mm, $r_3 = 5.0$ mm.

Rhodamine B and 1,1′-diethyl-2,2′-quinocyanine were used as sensitizing dyes. As supersensitizers, hydroquinone and potassium ferrocyanide were employed. The electrolyte was a 0.2 *M* Na_2SO_4 solution. All the chemicals used were of reagent grade.

A rotating ring-disk electrode system Type RRDE-1 and a dual potentiogalvanostat Type DPG-1 (Nikko Keisoku Co.) were used for the measurements.

The light source was a 500-W xenon lamp, and the wavelength of the illuminating light was selected with the use of a monochromator or colored glass filters. The illumination of the CdS electrode–electrolyte interface was transmitted through the bulk of the CdS electrode.

In the absence of sensitizing dyes in the electrolyte solution, only a small photocurrent can be observed with the illumination of light in the wavelength range above 540 nm, since the forbidden band width of CdS is about 2.4 eV. When rhodamine B was added into the electrolyte solution, a disk photocurrent, whose spectral distribution is quite close to the absorption spectrum of the dye adsorbed on CdS, was obtained as shown in Figure 2. In this case, no reduction current at the Au ring electrode was observed. This fact presumably suggests that the oxidation product of rhodamine B in the course of the sensitization process is of quite irreversible nature or is extremely unstable.

When hydroquinone was added to the dye-containing electrolyte solution, the photocurrent in the region of dye absorption was remarkably enhanced (supersensitization) as shown in Figure 2. When the potential of the Au ring electrode was fixed at, e.g., −0.4 V vs SCE, a reduction current, which was approximately proportional to the sensitization current at the CdS disk electrode, was detected at the ring electrode (broken curve in Figure 2).

In order to identify the species undergoing reduction at the ring electrode, the current-potential characteristic of