Magnetic Circular Dichroism Studies of Non-Iron "Hyper" Porphyrin Complexes as Models for Reduced + CO Cytochrome P-450[1a]

Sir:

The single most characteristic spectral feature of cytochrome P-450 is its intense 450-nm "reduced + CO" absorption band (Figure 1). Indeed, it was this feature which first led to the discovery of P-450[2] and to its name.[3] Recently a hypothesis was proposed[4] which accounts for the abnormal spectroscopy of this state of the protein by invoking an orbital mixing mechanism previously put forth to explain the equally unusual spectral properties of the so-called "hyper" porphyrins. The absorption spectra of metalloporphyrin complexes have been classified as normal, hypso, and hyper.[5] The normal porphyrins show well-characterized π–π^* visible, Soret, and near-UV transitions in the 700–300-nm region. Hypso porphyrins exhibit blue-shifted transitions. The characteristic feature of hyper porphyrins is the appearance of extra strong absorption bands. The principal finding presented in this communication is that the magnetic circular dichroism (MCD) spectra of the now well-understood hyperporphyrins are remarkably similar to that of reduced + CO cytochrome P-450. In light of the previously established[6] utility of MCD spectroscopy as a probe of the active site structure of cytochrome P-450 and because of its ability to more fully characterize electronic transitions, we feel that our present experimental findings provide strong support for the orbital mixing mechanism.[4]

Cytochrome P-450 has been the object of extensive scrutiny over the past 2 decades because of its unique enzymatic and spectroscopic properties.[7] It has the ability to activate coordinated dioxygen for insertion into a wide variety of organic compounds, including such unactivated substrates as cyclohexane. Spectrally it is unusual because in its reduced + CO state it exhibits a Soret absorption band at about 450 nm, approximately 30 nm red-shifted from the position of the corresponding absorption band of the more normal heme proteins such as hemoglobin and myoglobin. Additionally, as shown in Figure 1, P-450 has an absorption band at about 365 nm with an integrated intensity equal to that of the 450-nm band.[4] These two conspicuous spectral features have recently been reproduced by ferrous porphyrin model complexes containing an axial *thiolate* ligand trans to the CO.[8,9] Ether, imidazole,[8] thiol, thioether,[8,9] alcoholate,[9,10] carboxylate, and disulfide[9] ligation fail to reproduce the characteristic "450-type" spectrum. Thiolate ligation has also been strongly implicated from model studies of both substrate-free and substrate-bound ferric P-450.[11]

While contributing extremely useful structural information about the active site of P-450, and in turn providing a more sound basis for mechanistic speculation, the identification of the thiolate ligand does not ipso facto explain the unusual spectral properties. An explanation for these properties does come from a comparison of iterative extended Hückel (IEH) calculations on the thiolate species with similar calculations on hyperporphyrins,[4] which like P-450 frequently show two Soret bands—one in the 350–380-nm region and the second in the 450–480-nm region. The IEH calculations show that two Soret bands can arise by strong mixing between in-plane allowed charge transfer transitions with the normal Soret (π,π^*) transition of similar energy. In the case of p-type hyperporphyrins, such as P(III), As(III), Sb(III), and Bi(III),[12] these charge transfer (CT) transitions are of $a_{2u}(np_z) \rightarrow e_g(\pi^*)$ character, where $a_{2u}(np_z)$ is predominantly a metal orbital and $e_g(\pi^*)$ a ring orbital. Depending on the relative amount of CT or Soret character, the band at 350 nm or at 450 nm may be the more intense (i.e., more Soret character). A CT transition

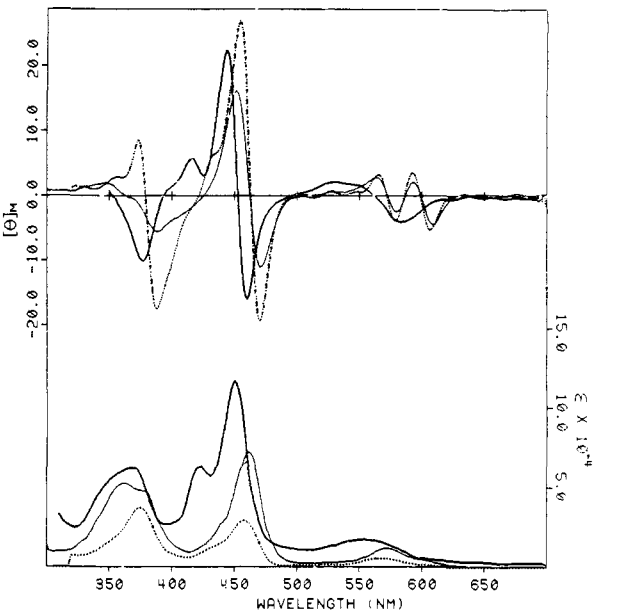

Figure 1. MCD and absorption spectra of purified reduced + CO $P\text{-}450_{LM_2}$ at pH 7.4 (heavy solid) and of (octaethylporphyrinato)antimony(III) chloride in ethanol (- - -) and (octaethylporphyrinato)bismuth(III) nitrate in CH_2Cl_2 (light solid).

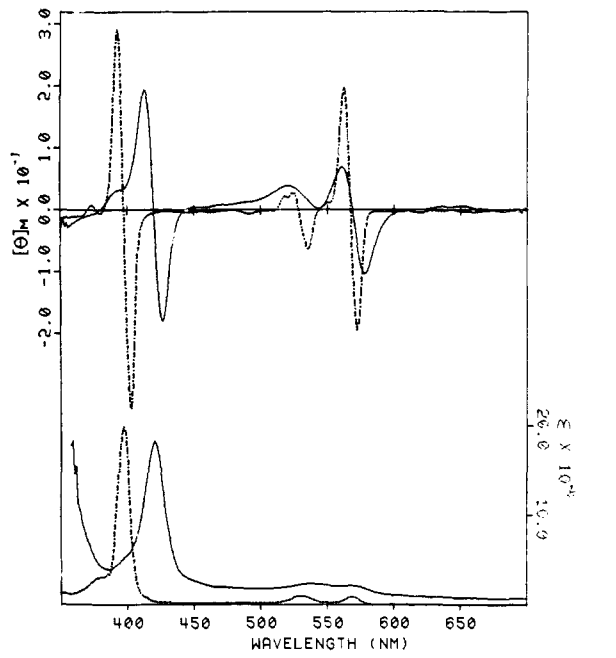

Figure 2. MCD and absorption spectra of purified reduced + CO P-420 at pH 7.4 (—) and (dihydroxo)(octaethylporphyrinato)antimony(V) chloride in CH_2Cl_2 (- - -).

from the thiolate ligand to the ring, $p^+(Sp) \rightarrow e_g(\pi^*)$, of similar symmetry is calculated to play a similar role in reduced + CO P-450.[4]

The classification of the spectrum of reduced + CO P-450 as "hyper" has only recently become clear due to the prevalent use of the near-UV absorbing reducing agent, sodium dithionite, which obscures the 360-nm porphyrin transition. The dual Soret nature of reduced + CO P-450[13] and of two typical hyperporphyrins, $(OEP)Sb^{III}Cl$[16] and $(OEP)Bi^{III}NO_3$[16] [OEP = octaethylporphyrin], are evident in their absorption[17] and MCD[18] spectra shown in Figure 1. The OEP model spectra are red shifted somewhat in wavelength, but still exhibit features corresponding to those of reduced +CO P-450 in the 500–340-nm Soret region. In particular, a derivative shaped MCD

effect is seen in the 450-nm band and a primarily negative effect is seen in the near-UV region. (The absorption and MCD spectra of P-450 exhibits impurity peaks at 415–420 nm due to the presence of a small amount of P-420 (compare Figure 2).) The visible portions of the model spectra show more fine structure perhaps due to the more symmetrical structure of OEP. Because MCD is a more sensitive measure of the electronic properties of chromophores, the similarity seen in both the shape and intensity of the MCD spectra of the well understood[4] group 5a hyperporphyrins and of reduced + CO P-450 lends credence to the proposal that their absorption spectra result from similar orbital mechanisms. We also note that the MCD spectrum of reduced + CO chloroperoxidase,[20] another protein which exhibits a "450-type" absorption spectrum, and of a reduced + CO model *thiolate* compound[6f] are nearly identical with that of reduced + CO P-450.

The rather different absorption and MCD spectra exhibited by the "normal" porphyrin $(OEP)Sb^{V}(OH)_2Cl$ and by reduced + CO P-420[21] shown in Figure 2 provide an interesting contrast to the spectra of the hyperporphyrins shown in Figure 1. As in the "hyper" spectra (Figure 1), the OEP model is somewhat shifted in wavelength relative to the natural porphyrin and shows more fine structure in the visible region. As discussed by Hanson et al.[4] the "normal" complexes lack the requisite conditions for a charge transfer transition, and thus no mixing is seen with the Soret π–π^* transition. The result is a single unshifted Soret absorption band and an uncomplicated MCD spectrum. (The absorption tail below 360 nm in the P-420 spectrum is due to dithionite.) The MCD of reduced + CO model heme complexes with thiol and imidazole trans to the CO are nearly identical with that of P-420.[6f]

The MCD data presented here provide strong support for the orbital mixing hypothesis of Hanson et al.[4] which has furnished, for the first time, a cogent explanation for the origin of the anomalous Soret spectrum of reduced + CO P-450.

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References and Notes

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(13) Reduced + CO P-450 is studied with mammalian $P\text{-}450_{LM_2}$[14] prepared by a modification of the method of Coon.[15] Exact details will be published elsewhere. The specific content of the $P\text{-}450_{LM_2}$ was approximately 17 nmol per mg protein.
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(17) Absorption spectra were recorded on a Cary 17 spectrophotometer at ambient temperatures. To avoid obscuring the 360-nm porphyrin transition, the reduced + CO P-450 spectrum was obtained through use of a minimal amount of sodium dithionite under anaerobic conditions. Such precautions are not necessary for MCD measurements because sodium dithionite shows only an extremely weak MCD effect in the 330-nm region.
(18) MCD measurements on the model complexes were made on a JASCO (Japan Spectroscopy Company) J-40 circular dichroism instrument using a 15 kG electromagnet.[19] The protein MCD spectra have been corrected for natural circular dichroism (MCD_{obsd} = MCD + CD). All data have been normalized and are expressed in the units of molar magnetic ellipticity, $[\theta]_M$, deg cm^2 dmol^{-1} G^{-1}. Measurements were made at ambient temperatures.
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(21) Cytochrome P-420 was obtained from cytochrome P-450 as described in ref 6f.

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Charge Directed Conjugate Addition. The Addition of Strong Nucleophiles to Unsaturated Acyl Ylides

Sir:

The addition of nucleophilic carbon centers to polarized carbon–carbon multiple bonds constitutes one of the fundamental processes for carbon skeleton construction. Such processes are often hampered by the simultaneous susceptibility of the polarizing moiety to attack by the nucleophile. This factor is especially important in additions to carbonyl-activated olefins where the dominance of conjugate addition over carbonyl addition is usually limited to cases involving relatively weak nucleophiles.[1] The discrete conjugate addition of strong nucleophiles to Michael type acceptors generally requires the use of organocopper reagents.[2] Methods less general in nature involve select donors and acceptors.[3]

It seemed plausible that discrete conjugate addition reactions might be possible in unsaturated carbonyl-deactivated systems such as **1** (Z = X^-) where additions would result in the formation of stable but reactive dianionic adducts **2** (Z = X^-). Dianions of this type are well known.[4] In such systems, direct 1,4-addition might be expected to predominate over carbonyl addition owing to the marked resistance of charge-