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Electronic Structure of Cytochrome *P450**

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The optical properties of *P450* have been investigated by means of polarized absorption spectroscopy of single crystals of camphor--bound *P450*CAM in the oxidized, reduced, and CO-reduced states, and iterative extended Hückel (IEH) calculations. The heme chromophores are orientated such that transitions polarized in the heme plane (*x,y*-polarized) can be readily distinguished from transitions polarized perpendicular to the heme plane (*z*-polarized). High spin oxidized *P450* exhibits two broad *z*-polarized bands, at 567 and 323 nm. IEH calculations suggest that these bands arise from cysteine mercaptide sulfur-to-iron charge transfer transitions. High spin reduced *P450* has no *z*-polarized bands. IEH calculations suggest that loss of these bands occurs because the cysteine sulfur is protonated to a mercaptan. Low spin CO-*P450* has an intense *x,y*-polarized band at 363 nm. This transition, assigned as a mercaptide sulfur-to-porphyrin charge transfer transition, has the correct symmetry to mix with the Soret and may cause the anomalous red shift of the Soret.

INTRODUCTION

The optical spectra of *P450* in several oxidation and spin states have been investigated in order to determine the valence electronic structure of the heme. These studies were undertaken with the rationale that an understanding of the heme electronic structure should eventually shed light on the chemical mechanism of *P450* hydroxylations. This paper will focus on new or distinguishing features in the *P450* spectra in comparison to the spectra of well characterized heme proteins. These features will be seen to depend on the nature of the heme axial ligands. The primary experimental technique employed was the absorption of polarized light by single crystals of *P450*CAM in the substrate-bound oxidized, reduced, and CO-reduced forms. The absorption spectra were interpreted with the help of iterative extended Hückel (IEH) semi-empirical molecular orbital calculations, a method which has been used extensively in the interpretation of metalloporphyrin electronic structure[1].

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An electronic transition is characterized by its intensity, energy, band width, and the direction of its transition moment relative to the molecular geometry. In an isotropic solution spectrum, only the first three parameters can be determined. In single crystals the chromophores are oriented, so that the transition moment directions can be determined as well. Crystals have the additional advantage that overlapping transitions with different transition moment directions can be sorted out. The *P450* chromophore, heme, is a planar absorber[2]; *i. e.* electronic transitions are polarized either parallel or perpendicular to the heme plane. If we define the porphyrin plane of heme as lying in the x, y plane and z as perpendicular to this plane, then the heme electronic transitions can be classified in the following manner:

1) The strong porphyrin $\pi \rightarrow \pi^*$ transitions, which dominate the isotropic solution spectra, are always x, y-polarized.
2) The weak iron d — d transitions may be either x, y- or z-polarized.
3) Likewise the moderate to weak »charge transfer« transitions between the porphyrin-iron, porphyrin-axial ligand(s), or axial ligand(s)-iron, may be either x, y- or z-polarized.

Thus any z-polarized transition which is observed in a polarized spectrum of an oriented heme protein should be due to a transition involving the iron and/or an axial ligand.

EXPERIMENTAL

Polarized absorption spectra were obtained with a recording microspectrophotometer[3] on single crystals of camphor-bound *P450*CAM from *P. putida*[4]. The crystals were grown from concentrated ammonium sulfate solutions at pH 7.0 (phosphate buffer). The native (ferric) crystals can be reduced with sodium dithionite and exposed to CO to produce the reduced and CO-reduced forms, respectively. Admission of oxygen to the system will rapidly reoxidize the crystals back to the high spin ferric state.

The iterative extended Hückel calculations were performed with a program provided by M. Gouterman[5]. The iron porphin model geometries for each oxidation, spin, and ligand state calculated were based upon relevant crystal structures[6].

RESULTS

The crystal face used for the optical measurements is illustrated in Figure 1. The crystal symmetry (for the ferric form) has been determined by R. E. Dickerson to be orthorhombic with four molecules per unit cell.[4] In an orthorhombic crystal, the principle optical directions coincide with the crystal axes. Since the accessible crystal face was the (001), the polarized spectra were taken with the electric vector of the incident radiation parallel to the a and b crystal axes respectively.

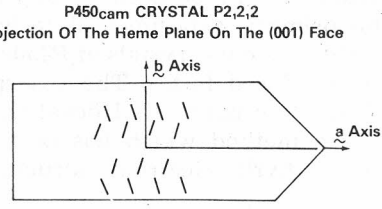

Figure 1. The (001) face of oxidized camphor-bound *P450*CAM crystals, space group $P2_12_12$. Polarized absorption spectra were taken with E ∥ a, b axes, respectively. The heavy black bar lines represent the approximate orientation of the edge-on heme planes.

Although the structure of these crystals has not been solved, the relative orientation of the heme planes can nonetheless be determined. We know from the space group symmetry that all the heme chromophores are optically identical. More importantly, the crystals are extremely dichroic with the major absorption occurring in the *b* axis direction. Thus the hemes must lie close to the *bc* crystallographic plane — *i. e.* perpendicular to the (001) face and nearly parallel to the *b* axis. This is a favorable situation because *x, y*-polarized transitions (polarized parallel to the heme plane) are readily distinguishable from *z*-polarized transitions: the *x, y*-polarized transitions will dominate the *b* axis spectrum while any *z*-polarized transitions will appear in the *a* axis spectrum.

The *a* and *b* axis polarized absorption spectra, the polarization ratio (PR = $= \varepsilon_b/\varepsilon_a$) from which one can calculate the heme plane orientation, and the solution spectra for the camphor-bound oxidized, reduced, and CO-reduced *P450*CAM are shown in Figures 2, 3 and 7, respectively. Figure 4 shows the »pure« *z*-polarized spectra for the oxidized and reduced forms, calculated by subtracting off the component of *x, y* intensity along the *a* axis direction. The major findings are as follows: 1) Two new *z*-polarized transitions of moderate intensity ($\varepsilon \sim 2$—3,000) have been discovered under the $\pi \rightarrow \pi^*$ manifold of the high spin oxidized *P450*CAM at 323 nm and 567 nm. 2) No new *z*-polarized bands are discernible in the high spin reduced *P450*CAM spectrum. 3) The low spin CO--reduced *P450*CAM has an intense *x, y*-polarized band at 363 nm and no *z*-polarized transitions. 4) The Soret band polarization ratio changes very little between the different oxidation and spin states (9.2 for high spin ferric to 10.8 for CO-ferrous). This indicates that only small changes in heme orientation ($\sim 1.2^0$) have taken place.

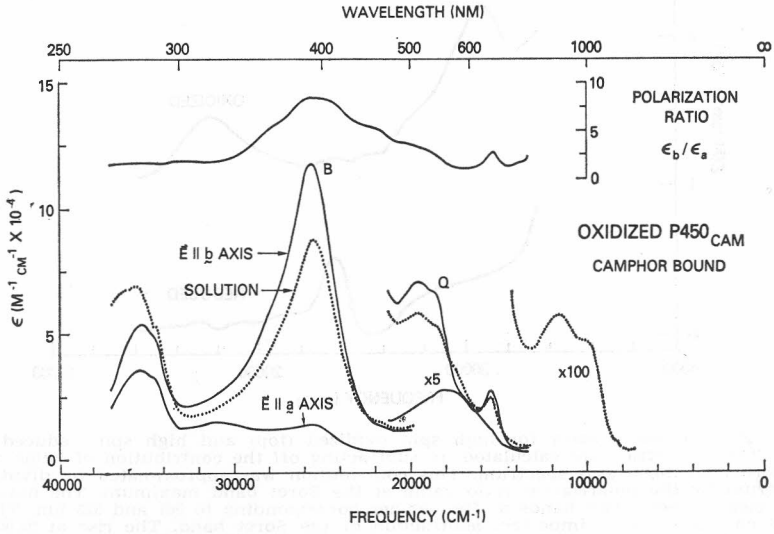

Figure 2. High spin oxidized *P450*CAM polarized single crystal absorption spectra (solid lines) and solution spectrum (dotted lines). The polarization ratio (top) is determined by dividing the *b* axis spectrum by the *a* axis spectrum. The B band is the Soret band, the Q bands are the visible or α, β bands.

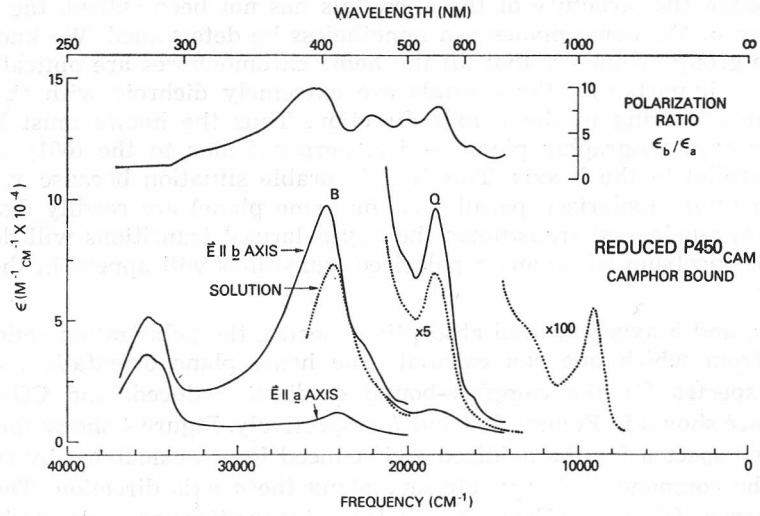

Figure 3. High spin reduced *P450*CAM polarized single crystal absorption spectra (solid lines), solution spectrum (dotted lines) and polarization ratio (top) in the (001) face.

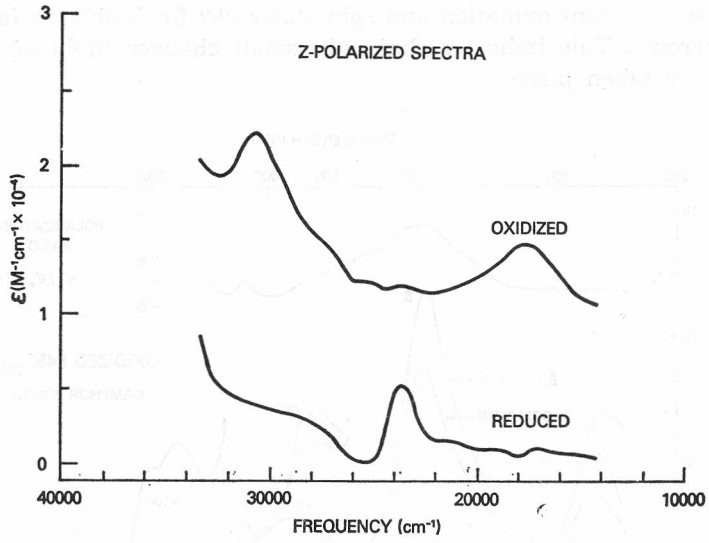

Figure 4. »Z-polarized« spectra for high spin oxidized (top) and high spin reduced (bottom) *P450*CAM. These spectra were calculated by subtracting off the contribution of heme *x*, *y*-polarized intensity to the *a* axis spectrum. This contribution was approximated by dividing the *b* axis spectrum by the polarization ratio value at the Soret band maximum. The oxidized *P450* spectrum clearly shows two bands at frequencies corresponding to 567 and 323 nm. The ripples at ~25 000 cm^{-1} are due to imperfect subtraction of the Soret band. The rise at 33 000 cm^{-1} is due the onset of amino acid absorption. In the reduced *P450* spectrum, the only observable features are a high baseline and a large distortion in the Soret region. This distortion represents the fact that the Soret maxima in the *a* and *b* axis spectra are displaced from each other by ~20 nm. A large displacement in Soret band maxima is also observed in single crystal spectra of deoxyHb.

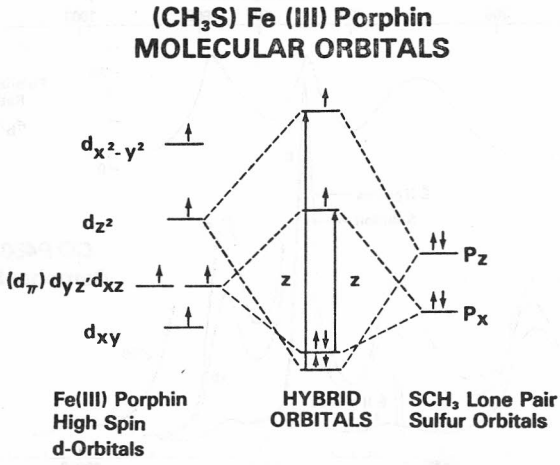

Figure 5. Results of the IEH calculation on high spin $(CH_3S)Fe(III)$porphin. The porphyrin π orbitals are not shown. On the left are the unperturbed iron d-orbitals, on the right, the unperturbed mercaptide lone pair sulfur orbitals, and in the middle, the result of orbital mixing.

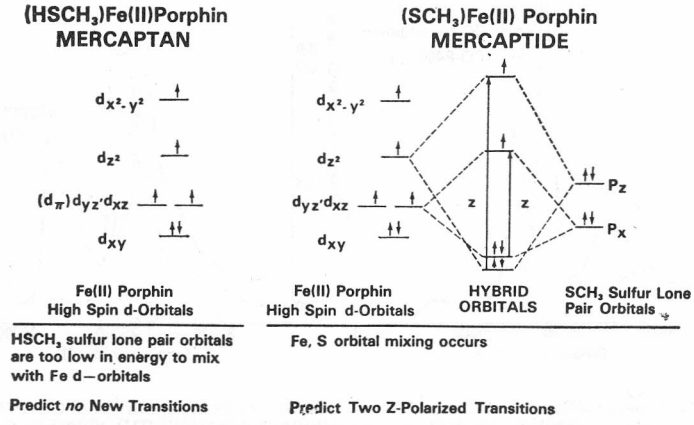

Figure 6. Results of the IEH calculations on mercaptan (left) and mercaptide (right) high spin ferrous porphin complexes. The porphyrin π-orbitals are not shown.

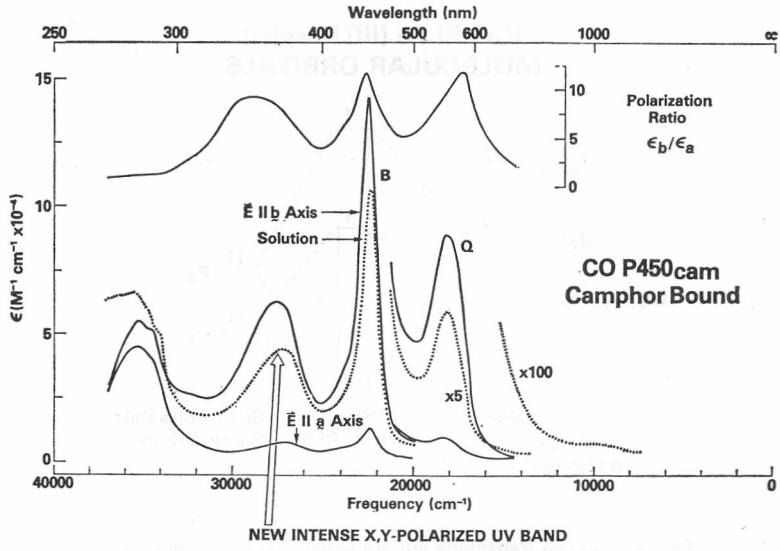

Figure 7. CO-*P450*CAM polarized single crystal absorption spectra (solid lines), solution spectrum (dotted lines) and polarization ratio in the (001) face.

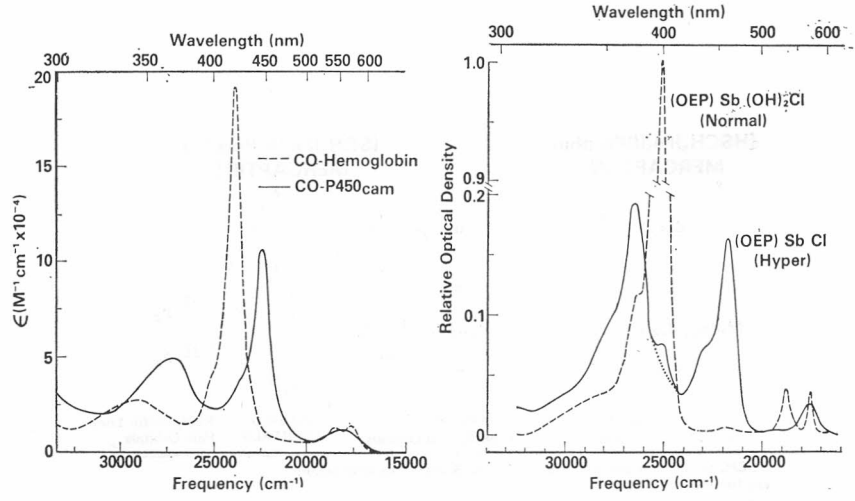

Figure 8. Left: Superposition of the solution spectra of CO-hemoglobin and CO-*P450*CAM. Right: Superposition of the absorption spectra of (octaethylporphinato)Sb(III) chloride (solid line) and its spontaneous oxidation product upon exposure to air, (dihydroxo)(octaethylporphinato)Sb(V) chloride (broken line) in dichloromethane at room temperature. The presence of Sb(V) impurity in the Sb(III) spectrum is denoted by the dotted line.

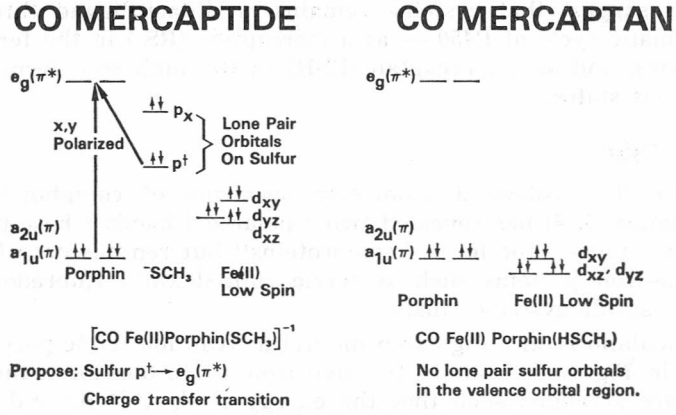

Figure 9. Results of the IEH calculations on low spin ferrous CO mercaptide (left) and mercaptan (right) porphin complexes. The empty iron d_{z^2} and $d_{x^2-y^2}$ orbitals are very high in energy and are not shown.

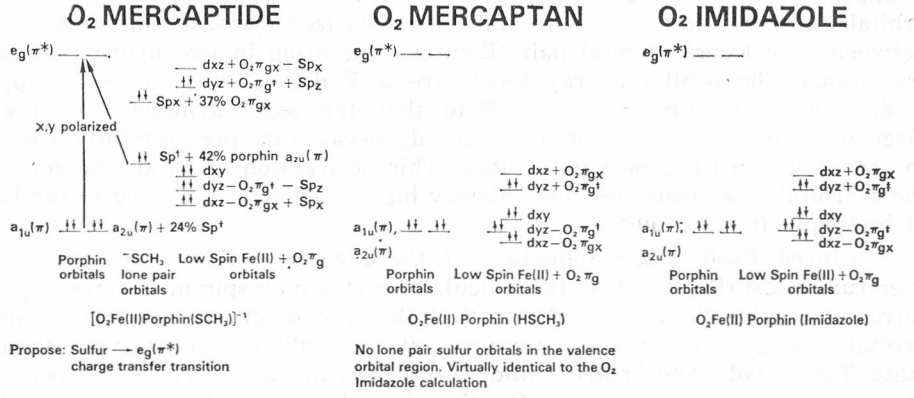

Figure 10. Results of the IEH calculations on low spin ferrous O_2 mercaptide (left), mercaptan (center) and imidazole (right) porphin complexes. In all these cases, the O_2 π^* orbitals and the iron d_π orbitals are strongly mixed, however, in the mercaptide case, lone pair sulfur orbitals are also present.

The results of the IEH calculations on various model compounds are shown schematically in Figures 5, 6, 9, and 10, and will be discussed under the interpretation of the spectral data.

DISCUSSION

When these studies were begun, there was still considerable doubt as to the nature of the axial ligand(s) to the *P450* heme, especially whether and to what extent cysteine played a role. There was evidence for a mercaptide sulfur (RS^-) ligand in the ferric state, based upon denaturation of the protein by sulfhydryl reagents[7], and comparisons of the EPR spectra with thiolated metmyoglobin derivatives.[8] During the last two years, EPR and optical comparisons with newly synthesized and characterized iron porphyrin model compounds have resulted in general agreement that the ferric[6a,9] and CO-ferrous[10] states of *P450* have mercaptide ligands. Our results support this view

and further suggest that cysteine remains an intact ligand throughout the entire enzymatic cycle of *P450* — as a mercaptide (RS^-) in the ferric and CO--ferrous states, and as a mercaptan (RSH) in the high spin ferrous (reduced) and O_2-ferrous states.

High Spin P450

Oxidized: The polarized absorption spectrum of camphor-bound ferric *P450*CAM (Figures 2, 4) has revealed two *z*-polarized bands whose positions and intensities are unique for ferric hemoproteins[11] but reminiscent of the spectra of non-heme-iron proteins such as ferric rubredoxin[12]. Rubredoxin contains an iron-mercaptide cysteine cluster.

IEH calculations on a high spin methyl mercaptide ferric porphin complex are shown in Figure 5. Usually the five iron d orbitals for a high spin iron porphyrin are arranged such that the energy of $d_{x^2-y^2} > d_{z^2} > d_{xz} = d_{yz} > d_{xy}$ and these orbitals are negligibly perturbed by axial ligands[13]. However, a mercaptide ligand apparently alters this picture drastically. Two lone pair sulfur p orbitals appear in the valence energy region of the iron and mix strongly with two of the half-filled iron d orbitals, creating four new hybrid orbitals: $d_{z^2} \pm p_z$ and $d_{xz} \pm p_x$. An allowed *z*-polarized transition may take place between each hybrid orbital pair (Figure 5) resulting in two new *z*-polarized transitions. The orbital energy levels are now $d_{x^2-y^2} > d_{z^2} - p_z > d_{xz} - p_x > > d_{yz} > d_{xy} > d_{xz} + p_x > d_{z^2} + p_z$. Note that the axial symmetry of the d electrons is broken. d_{yz} no longer equals d_{xz} because d_{xz} preferentially π-bonds to one of the sulfur lone pair orbitals. This destruction of axial symmetry of the d orbitals may cause the anomalously high rhombicity observed in the EPR of high spin ferric *P450*[14].

Reduced P450: Upon reduction of the high spin *P450,* the *z*-polarized spectrum is lost (Figures 3, 4). IEH calculations on a high spin methyl mercaptide ferrous porphin (Figure 6) indicate that the iron d and mercaptide sulfur p orbital mixing which occurs in the ferric state should also occur in the ferrous state. Thus *z*-polarized bands should be observed in the reduced *P450* spectrum if mercaptide is still a ligand. On the other hand, a calculation on methyl mercaptan (CH_3SH) ferrous porphin indicates that this complex should have a »normal« spectrum with *no* extra transitions. The d orbitals are unperturbed since the mercaptan lone pair sulfur orbitals are too low in energy to mix with the iron d orbitals. Reduced high spin *P450* may well have a mercaptan cysteine as its axial ligand.

The loss of the *z*-polarized bands upon reduction can also be explained in terms of axial ligand exchange, *i. e.,* the replacement of the cysteine by another amino acid residue. This explanation seems highly unlikely in light of two half-filled d orbitals with two lone pair cysteine mercaptide sulfur p reduction (deduced from the Soret band polarization ratios)[15]. It is difficult to imagine that an exchange of ligands to the heme, where both potential ligands are bound to the protein as amino acid residues, would not result in appreciable heme plane orientation changes. However, addition of a proton to a cysteine mercaptide sulfur, perhaps donated by a nearby amino acid residue, should leave the heme orientation intact.

In conclusion, the two *z*-polarized bands observed for high spin ferric *P450* can be assigned as transitions between hybrid orbitals formed by the mixing

of two hal-filled d orbitals with two lone pair cysteine mercaptide sulpuhr p orbitals. Upon reduction, a proton as well as an electron may be added to the *P450* heme complex, resulting in a cysteine mercaptan-ferrous complex. This mercaptan complex is predicted to give no extraneous transitions, and, indeed, a *z*-polarized spectrum is not observed for the reduced *P450*.

Low Spin P450

CO-Ferrous: The single most characteristic and distinguishing optical feature of *P450* is the Soret maximum at approximately *450* nm in the CO--reduced form. This Soret band occurs at wavelengths 30 nm longer than the usual CO-heme complex. The single crystal absorption spectrum (Figure 7) shows, in addition to the red shifted Soret band (at 446 nm in *P450*CAM), a prominent uv band at 363 nm which has the same polarization and integrated intensity as the Soret. We have presented in a previous publication[16] an interpretation of the »anomalous« electronic spectrum of CO-*P450*. It was proposed that the intense uv and red shifted Soret bands in CO-*P450* are due to an allowed mercaptide sulfur to porphyrin charge transfer transition which strongly mixes with the Soret $\pi \rightarrow \pi^*$ transition, borrowing its intensity and shifting it to longer wavelengths. The reasoning behind this assertion will be briefly reiterated.

The crystal spectrum shows that the 363 nm band and the Soret band have the same symmetry since they are both *x, y*-polarized. Furthermore, the 363 nm band has most probably gained its intensity by borrowing it from the Soret. In Figure 8 left, a superposition of the solution spectra of CO-*P450*CAM and carboxyhemoglobin (COHb) is shown. The total integrated intensity over the CO-*P450* 363 nm and 446 nm bands agrees to within 10% of the total integrated intensity over the N (345 nm) and Soret (421 nm) bands of COHb. Thus the total integrated intensity is conserved between CO-*P450* and COHb.

There are two classes of metalloporphyrins, known as *d-type* and *p-type* hyperporphyrins[16], which also give anomalous Soret spectra, *i. e.* one intense band in the 350—380 nm region and another in the 440—480 nm region. The *d-type* hyperporphyrin, of which Cr(III)[17] and Mn(III)[18] are examples, contain metals with vacancies in the $e_g(d_\pi)$ orbitals. Since the d_π orbitals of ferrous CO-*P450* are filled, the *d-type* hyperporphyrins will not be discussed further. The *p-type* hyperporphyrins contain main group metals in lower oxidation states such as Sn(II), Pb(II)[19], P(III)[20], As(III), Sb(III), and Bi(III)[1b]. An example for comparison with CO-*P450* is shown in Figure 8 right. The Sb(III) porphyrin, with a filled 5s metal orbital, gives an anomalous spectrum. Upon spontaneous air oxidation to an Sb(V) compound, in which the 5s electrons are lost, the spectrum looks like a normal porphyrin spectrum. The integrated absorption strength between the Sb(III) and Sb(V) is conserved to within 6%. IEH calculations by Gouterman and co-workers[16,19a] on *p-type* hyperporphyrins indicate that a charge transfer transition from the lone pair metal ns orbital to the porphyrin $e_g(\pi^*)$ mixes with the Soret $\pi \rightarrow \pi^*$ and shares its intensity, thereby causing the intense uv and red-shifted Soret bands. The key to the spectral anomaly is thus the presence of a lone pair orbital from which a suitable charge transfer transition might be made into the empty porphyrin $e_g(\pi^*)$ orbitals. We now look for such a transition in CO-*P450*.

IEH calculations on methyl mercaptide ferrous CO-porphin and methyl mercaptan ferrous CO-porphin are shown schematically in Figure 9. Synthesis of *P450* model compounds has shown that mercaptide (RS^-) CO-ferrous porphyrin complexes have hyper spectra[10c,d] whereas mercaptan (RSH) CO-ferrous complexes have Soret bands at »normal« wavelengths (413—422 nm)[10a,b]. The calculations show that the mercaptide complex, but not the mercaptan complex, has two lone pair sulfur orbitals in the porphyrin valence region, below the porphyrin $e_g(\pi^*)$. One of these, labelled p†, has the correct symmetry for an allowed sulfur p† $\rightarrow$ $e_g(\pi^*)$ charge transfer transition. (The lone pair mercaptide sulfur orbitals do not mix with the low spin ferrous iron orbitals, contrary to the mixing that occurs with the high spin ferric/ferrous iron orbitals). Thus we postulate that the intense uv and red-shifted Soret bands observed in both the model compounds and CO-*P450* are due to a mercaptide sulfur to porphin charge transfer transition which mixes strongly with the Soret $\pi - \pi^*$ transition.

Other P450 Ferrous Low Spin Complexes

Our orbital mechanism for the origin of hyper spectra in CO-*P450* and the CO model compounds leads to the prediction that other low spin ferrous mercaptide complexes will exhibit hyper spectra whereas low spin ferrous mercaptan complexes will exhibit normal spectra. Published absorption spectra of O_2-*P450*[4,21] (before the addition of the 2nd electron) are similar to O_2Hb with a Soret maxima at 418 nm. The IEH calculations on O_2 mercaptide, mercaptan, and imidazole ferrous complexes are shown in Figure 10. Identical orbital patterns are calculated for the imidazole and mercaptan complexes in contrast to the mercaptide complex, which has extra lone pair sulfur orbitals below the porphyrin $e_g(\pi^*)$ in a manner analogous to the CO mercaptide[22]. Thus we predict on the basis of the IEH calculations, that O_2 mercaptide heme complexes can exhibit hyperspectra and O_2 mercaptan heme complexes will exhibit »normal« spectra. Therefore ferrous O_2-*P450* may well be a cysteine mercaptan complex.

Ferrous *P450* complexes with ligands such as ethylisocyanide or metyrapone[23] are known to exhibit spectra with Soret maxima at both $\sim$ 450 nm and $\sim$ 425—430 nm. The ratio of the 450/430 nm peaks increases with increasing pH. We can predict on the basis of the foregoing discussion that the cysteine ligand is partially protonated when these ligands are bound, *i. e.*, the spectra are due to a mixture of the mercaptide and mercaptan forms. The amount of mercaptide present should increase with increasing pH.

CONCLUSION

A summary of the major experimental findings of this work and their interpretation is presented in Figure 11. In general a mercapti*de* cysteine axial ligand to the heme gives rise to new transitions in the optical spectra, as in high spin oxidized and CO-*P450,* because of the presence of lone pair sulfur orbitals in the heme valence electron region. A mercapt*an* cysteine ligand should not perturb the heme spectrum because its lone pair sulfur orbitals are too low in energy to interact, therefore, both high spin reduced and O_2-*P450* may have a mercaptan cysteine ligand.

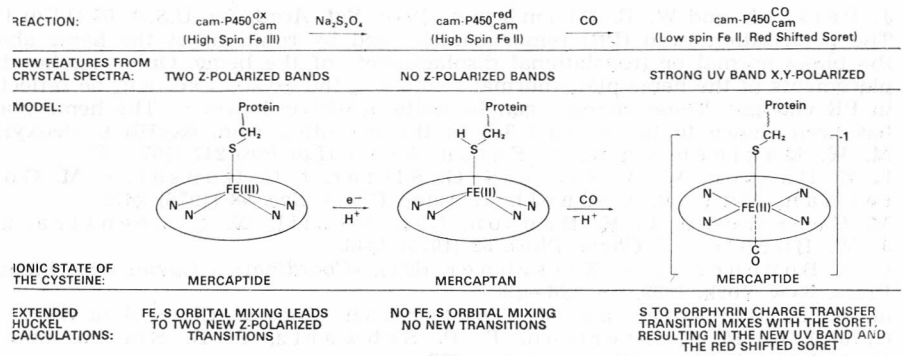

Figure 11. Summary of the single crystal results and their interpretation.

Acknowledgement. — The optical measurements were performed in the laboratory of Dr. William A. Eaton. We thank him for his guidance and many helpful discussions. We also thank Prof. Martin Gouterman for providing the IEH program and for insight into the interpretations of the low spin ferrous calculations.

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DISCUSSION

R. H. Austin:

a) Why isn't the polarization ratio constant as opposed to the large variations seen with wavelength?

b) Does the integrated peak area for *P450*CAM^{ox} = Hb^{ox} (met hemoglobin)?

L. K. Hanson:

a) 1. Below 300 nm the spectrum is dominated by the aromatic amino acid absorptions. The total dichroism resulting from a summation over all these residues is much lower than that of the edge-on heme.
2. Some of the variations in polarization ratio (PR) above 300 nm reflect the presence of *z*-polarized transitions as in substrate-bound oxidized *P450*CAM.
3. An error in positioning the baseline will be reflected to a much greater extent in the weakly absorbing direction than in the strongly absorbing direction, especially off the absorption peaks. This can result in dips in the PR between the absorption peaks.

b) I haven't compared them.

K. Ruckpaul:

Do you suppose that the position of the short wavelength shifted component of the first band in the CO-complex of *P450* is dependent on the ligand? We have the feeling from CD measurements that there is a ligand dependent difference. Would this fit with your calculations?

L. K. Hanson:

Yes. The positions and relative intensities of both components of the »split« Soret band of the CO complexes should depend on the energy of the lone pair mercaptide sulfur orbitals and their degree of overlap with the porphyrin π orbitals. This will depend on the geometry of the binding of the cysteine ligand of *P450*, the identity of R in (RS^-) CO-ferrous model compounds, and the polarity of the environment.

H. Rein:

a) Can you explain to me the charge transfer from the axial ligand to the porphyrin?
b) Hybridization of the sulfur — what is the angle used in the calculations?
c) I think the degeneracy of d_{yz}, d_{xz} in Fe^{3+} in *P450* is not real!

L. K. Hanson:

a) The charge transfer takes place from a lone pair mercaptide sulfur p orbital to the porphyrin in the case of low spin ferrous mercaptide complexes. The mechanism is explained in the text.

b) The calculations were performed with Fe-S-C angles of 120 and 109 degrees. The results are qualitatively the same with either angle.

c) I did not say that they were degenerate. My results show unequivocally that $d_{xz} \neq d_{yz}$ for both high spin ferric and high spin ferrous mercaptide complexes.

E. Sackmann:

You attribute the rather strong *z*-polarized bands to S-porphyrin charge transfer bands. Do you have any explanation or theoretical estimation showing why the CT-transitions should have such a high transition moment?

L. K. Hanson:

The *z*-polarized bands are observed in the high spin ferric *P450*. These are due to transitions between hybrid orbitals formed by mixing of iron d and mercaptide sulfur lone pair p orbitals (see text). The porphyrin π orbitals are not involved. Although I have not calculated transition moments for these transitions, I estimate them to be fairly large because the transitions are symmetry allowed, the degree of orbital overlap is large, and the charge distribution in the molecule remains unchanged by the transition.

P. Debey:

a) Could it be that the so-called »reversible« *P420* found with both microsomal *P450* and camphor *P450* could be due to a protonation of the cysteine which would turn to a mercaptan?

b) Do you think that the near ir region of the optical spectra could tell something concerning the formal charge of the iron, as for example a distinction between Fe^{3+} and Fe^{4+}?

L. K. Hanson:

a) It seems possible.

b) Perhaps. One would have to build up a library of near ir spectra for known Fe^{4+} complexes and compare them to the near ir spectra of Fe^{3+} complexes to see if there are any major differences. In general, high spin and low spin Fe^{3+} complexes do have distinguishing spectra in this region.

I. C. Gunsalus:

Are you inclined toward H_2O as 6th ligand in low spin *P450*CAM? Fast proten exchange from NMR measurements have been interpreted to so imply.

L. K. Hanson:

No, not in the camphor-free form.

R. H. Austin:

a) Do the calculations give any reason why the CO bound *P450* should be a mercaptide as opposed to a mercaptan?

b) It seems a good way to look for this mercaptan-mercaptide exchange would be to photo-dissociate CO and look for transient states either in the reduced state or perhaps a transient mercaptan-CO state after rebinding.

L. K. Hanson:

Not really. Perhaps a significant difference might be seen if I were to calculate the C ... Fe bond strength for the CO mercaptide and mercaptan complexes.

T. G. Traylor:

You suggest that, in *P450*CAM, CO prefers mercaptide over mercaptan as a sixth ligand whereas oxygen prefers mercaptan over mercaptide. This seems to be contrary to experience where electron donating sixth ligands increase oxygen binding but not CO binding. Is there an explanation for this?

L. K. Hanson:

As to why CO prefers mercaptide and O_2 prefers mercaptan, you have hit on a contradiction in the calculations. The calculated acidities for the mercaptan proton follow this order: $O_2 > CO \simeq$ high spin ferrous. I think one can safely surmise that the presence or absence of the 6th ligand and its identity will affect the pk_a of the mercaptan proton. I don't know what the affinity of O_2 binding to *P450* is relative to hemoglobin or myoglobin, but with regards to CO, CO binds to *P450* with a much lower affinity than to hemoglobin. This does not contradict your statement that electron donating 6th ligands decrease CO binding, since mercaptide is more electron donating than histidiene.

I. C. Gunsalus:

Certainly the CO dissociation is higher with the ferrous *P450* than hemoglobin or myoglobin. With *P450*CAM the K_D for O_2 and CO are nearly the same, in the micromolar range.

H. Schleyer:

1) We have measured these »double bands« of $Fe^{2+} \cdot L$ complexes for a variety of N-bases — with pharmaceutical products especially — where we also find often the analogous absorption for the 430 and 450 nm bands of the isocyanides. No such double bands, however, with »potential radical-forming agents« such as halothane, CCl_4 — which give only one broad absorption at $450 \geq \lambda \geq 475$. The combination of these latter absorptions with corresponding near uv bands (range ~ 360) and corresponding shifts are seen.

2) Have you investigated the epr experiments on »model systems« *i. e.* Röder, Williams, *et. al.* in terms of RSH versus RS^-? That seems necessary in terms of your findings, since these early models were all made with heme + RS*H* in large excess.

L. K. Hanson:

2) This is a good point. No, I haven't. However, whether they had RSH or RS^- as ligands to the heme would depend to a large extent on the pH of their systems.

SAŽETAK

Elektronska struktura citokroma *P450*

L. K. Hanson, S. G. Sligar i I. C. Gunsalus

Optička svojstva citokroma *P450* iz *Pseudomonas putida* ispitivana su s pomoću polarizirane apsorpcijske spektroskopije monokristala citokroma *P450* s vezanim kamforom u oksidiranom, reduciranom stanju, i u reduciranom s CO, uz primjenu iterativne proširene Hückelove metode računanja (IEH). Kromofori hema orijentirani su tako da se prijelazi polarizirani u ravnini hema (*x,y*-polarizirani) mogu lako razlučiti od prijelaza polariziranih okomito na ravninu hema (*z*-polarizirani). Visokospinski oksidirani *P450* ima dvije široke *z*-polarizirane vrpce, na 567 i 323 nm. Računanje prema IEH ukazuje da te vrpce potječu od prijelaza zbog prijenosa naboja od merkaptidnog sumpora cisteina na željezni ion. Visokospinski reducirani *P450* nema *z*-polariziranih vrpci. Račun IEH pokazuje da tih vrpci nema zato što je cisteinski sumpor protoniran u merkaptan. Niskospinski CO-*P450* ima intenzivnu *x,y*-polariziranu vrpcu na 363 nm. Taj prijelaz, koji se pripisuje prijenosu naboja s merkaptidnog sumpora na porfirin, ima ispravnu simetriju da bi se miješao sa Soretovom vrpcom i tako uzrokovao anomalni crveni pomak Soretova maksimuma.

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