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THIOL-CONTAINING PEPTIDE-HEMIN COMPLEXES AS MODELS OF CYTOCHROME P-450

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Summary: Nine different synthesized thiol tetra- and penta-peptides containing Cys-Ser, Cys-Thr, Cys-His, Cys-Tyr and Cys-Cys in the N- and C-terminals are proposed as new models of apo-P-450. The peptide-hemin complexes in the oxidized (Fe(III)) form in solution were characterized by their optical and EPR spectra and found to show hydroxylation activity like that of P-450 enzymes on acetanilide. Although the EPR properties of the complex containing Cys-His and all complexes in the presence of pyridine were similar to those of P-450, the optical properties of these complexes were not completely similar to those of P-450. Based on these results, the sixth heme coordination site of P-450 was discussed.

Cytochrome P-450 catalyses oxidation of a wide variety of compounds including drugs, chemical carcinogens, steroids and fatty acids (1,2). This group of P-450 enzymes is widely distributed in living things ranging from bacteria to higher animals (1). In general, the optical and magnetic properties of P-450 species indicate that these species all have a very similar structure around the heme binding site (1). A thiolate ligand in the 5th coordination position of the heme has been postulated to be essential for these properties (3). Recent studies have suggested that the 6th position is occupied by oxygen of a hydroxyl group of either Ser, Thr or Tyr residue or of water rather than by nitrogen of a His residue (4-7). Models may be useful in investigating this

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Abbreviations : S, H-Cys-Ala-Gly-Ser-OH; T, H-Cys-Ala-Gly-Thr-OH; H, H-Cys-Ala-Gly-His-OH; AS, H-Cys-Ala-Gly-Ala-Ser-OH; AT, H-Cys-Ala-Gly-Ala-Thr-OH; AH, H-Cys-Ala-Gly-Ala-His-OH; Y, H-Cys-Ala-Gly-Tyr-OH; AY, H-Cys-Ala-Gly-Ala-Tyr-OH; AC, H-Cys-Ala-Gly-Ala-Cys-OH; py, pyridine; Im, imidazole; GSH, glutathione; Hm, hemin; AAP, acetaminophenol; P-450, cytochrome P-450; EPR, electron paramagnetic resonance.

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problem. We postulated in the proceeding papers (8-14) that the thiol group of the ligand coordinated to heme iron is essential for the appearance of the characteristics of P-450 as well as for hydroxylating activity on substrates. Although many efforts have been made to construct models of P-450 (15), few thiol-containing peptide-heme complexes that can simulate both the spectral properties and hydroxylating activity of P-450 have been reported (10-12). We synthesized nine thiol tetra- and penta-peptides containing Cys-Ser, Cys-Thr, Cys-His, Cys-Tyr and Cys-Cys in the N- and C-terminals as models of apo-P-450. This paper reports the characterizations of these peptide-hemin complexes in the oxidized (Fe(III)) form in solution and their activity in aromatic hydroxylation. These complexes are proposed as possible models of P-450.

Materials and Methods

Peptides were synthesized in our laboratory as will be described elsewhere (16). Hemin(type I, bovine) was obtained from Sigma Chemical Co. Optical spectra were measured with a Union SM-302 spectrometer at room temperature, EPR spectra were recorded on glass at 77K with a JES-ME3X spectrometer operated at 100 KHz. As standards, DPPH powder and MgO powder doped with Mn(II) were used. According to Bohan's method (17), ligand field parameters of axial distortion and rhombic distortion at the Fe coordination sphere of low spin peptide-Hm complexes were computed from the g-values. Hydroxylation of acetanilide with the model compounds was assayed as reported (18).

Results and Discussion

Photometric acid-base titration of a complex consisting of AH and Hm at room temperature indicated that only a ferric complex in the high-spin state (469, 391, 506 and 629 nm) was formed in the acidic pH region, whereas ferric complexes in the high- and low-spin states (362, 413, 539, 566 and 633 nm) were both formed at pH 6.8-10.5. The py-titrated spectral change of the system at pH 9.6 was reversible and showed four isobestic points at 390, 450, 500 and 584 nm, indicating that the two chemical species were in equilibrium (Fig. 1). The corresponding EPR spectra of the AH-Hm complex (Fig. 2) resemble those of P-450 (1, 19) and model complexes (20, 21) that retain a thiolate-Hm coordination bond. The high-spin signal (g=8.4) in the presence of py may be that of a five coordinate thiolate-Hm complex, judging from the g-values reported previously (19,22). The g-values due to the formation of ferric low-spin complexes in the absence and presence of py are clearly different. Presumably the former is that the com-

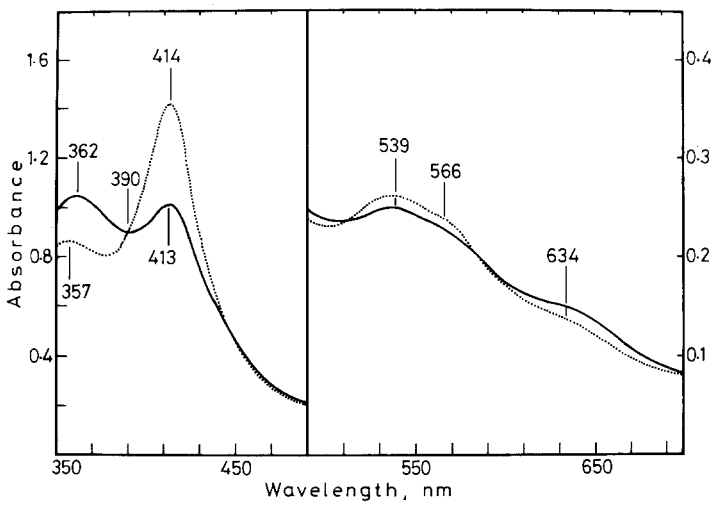

Figure 1. Optical spectra of the AH-hemin complex at pH 9.6 and room temperature. (——): AH 6.57 mM + hemin 25 μM, (------): AH 6.57 mM + hemin 25 μM + py 206 mM.

plex including the intramolecular peptide(S^-)-Hm-(N)Im and the latter is that of the peptide(S^-)-Hm-py coordination mode. The optical spectrum of the AS-Hm complex prepared at pH 9.6 showed one more absorption band near 442 nm (Fig. 3),

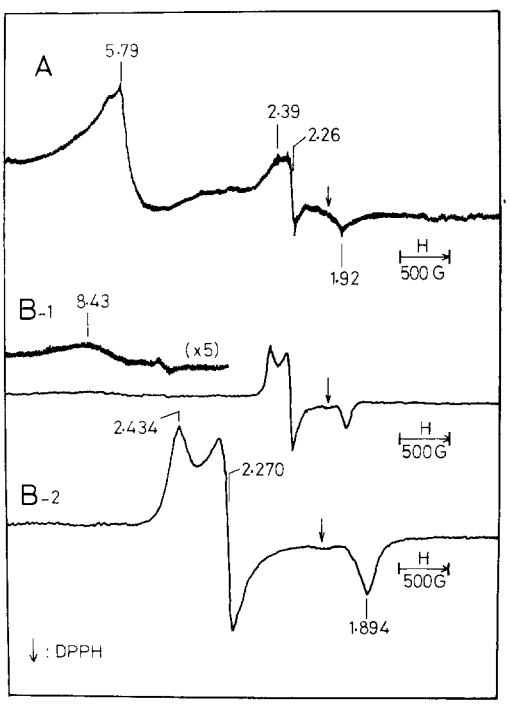

Figure 2. EPR spectra of the AH-hemin complex at pH 9.6 and 77K. A: AH 258 mM + hemin 5 mM, B: AH 258 mM + hemin 5 mM + py 2.1 M.

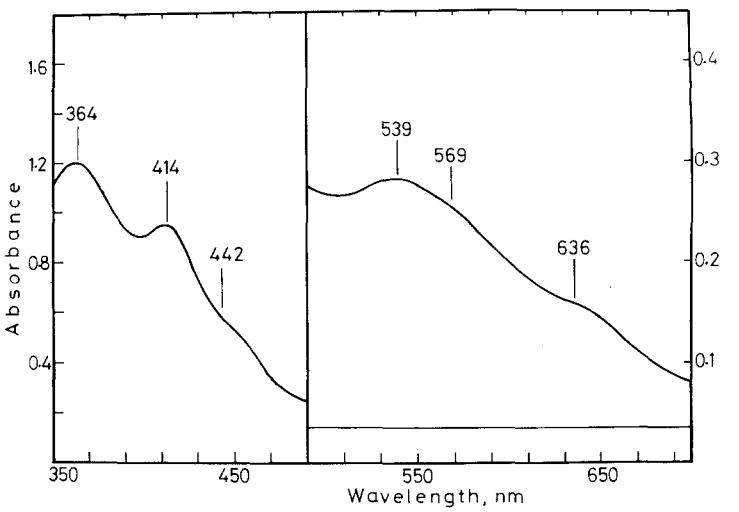

Figure 3. Optical spectrum of the AS-hemin complex at pH 9.6 and rrom temperature. The concentrations of AS and hemin are 7.36 mM and 25 μM, respectively.

suggesting the formation of a small amount of dithiolate-Hm complex consisting of 2 mol of peptide and Hm (23, 24).

The optical and EPR spectral properties of all the peptide-Hm complexes are summarized in Tables I and II, respectively, with those of P-450 enzymes

Table I. Spectral properties of peptide-hemin complexes at room temperature

peptide	pH	λ max (nm) peptide + hemin						λ max (nm) peptide + hemin + py					
S	7.5	364	412		531	566s	637s	362	412	535	547s	568s	637s
	9.6	365	414	441s	543	570s	629s	356	415	541		565s	635s
T	7.5	366	410		510		639s	355	414	534		565s	629s
	9.6	365	410s	442s	537	571	636s	356	414	540		565s	629s
H	7.5	365	414		534	566s	636s	360	414	537		564s	631s
	9.6	364	414		538	569s	633s	358	415	540		565s	631s
AS	7.5	363	412		531	566s	639s	361	413	534		569s	637s
	9.6	364	413	442s	539	569s	636s						
AT	7.5	362	412		531	566s	636s	361	414	536	548s	569s	638s
	9.6	363	411	444s	540	568s	639s	361	413	538		568s	639s
AH	7.5	361	414		534	566s	635s	359	414	536		568s	637s
	9.6	362	413		539	566s	633s	357	414	539		566s	634s
Y	7.5	365	419		538	568s	639s	358	418	538		568s	635s
	9.6	364	419		540	568s	642s	359	418	539		566s	634s
AY	7.5	367	417		534		647s	358	417	539		566s	634s
	9.6	367	417	443s	543		640s	358	417	539		569s	634s
AC	7.5	366	415		531		642s	358	417	537		568s	638s
	9.6	366	417	443s	541		638	357	417	535		554s	636s
ox-P450-camphor[a]													
high-spin		391		520	540		645						
low-spin			417		535	571							
ox-P450-liver microsomes[a]													
high-spin		394		517	540s								
low-spin			417		534	568							

[a] references 19, 20 and 22.

Table II. Principal g-values of EPR spectra of peptide-hemin complexes at 77K

Ligands bound to hemin	pH 7.4 No.	g-values				pH 9.6 No.	g-values				
no ligand		5.76					5.77				
S + py (0.5 M)	1	8.16	4.19	2.424	2.268	1.898		5.85				
S + py (2.0 M)	2			2.418	2.269	1.903	13	8.58		2.43	2.26	1.89
T		5.84	4.18					5.84				
T + py (2.0 M)	3		4.18	2.425	2.269	1.910	14	8.51	5.82	2.43	2.265	1.897
H		5.90	4.18				15	5.82		2.39	2.26	1.92
H + py (2.0 M)	4			2.41	2.27	1.90	16	6.01		2.43	2.270	1.899
				2.36	2.25	1.92						
AS		5.87	4.17				17	5.88		2.41	2.26	1.93
AS+ py (2.0 M)	5	6.01		2.42	2.27	1.91	18			2.43	2.27	1.90
AT		5.87					19	5.83		2.39	2.26	1.92
AT+ py (2.0 M)	6	7.79		2.414	2.267	1.908	20			2.435	2.271	1.899
AH		5.87					21	5.79		2.39	2.26	1.92
AH+ py (2.0 M)	7			2.423	2.267	1.902	22	8.43	4.18	2.434	2.270	1.894
				2.38	2.25	1.92						
Y		5.88						5.89				
Y + py (1.0 M)	8			2.425	2.271	1.893	23			2.438	2.273	1.894
Y + py (2.0 M)	9	5.93		2.385	2.271	1.914	24			2.379	2.269	1.922
AY		5.89										
AY+ py (2.0 M)	10	5.95		2.431	2.274	1.897	25			2.442	2.275	1.892
AY+ py (4.0 M)							26			2.37	2.27	1.92
AC		5.98						5.96				
AC+ py (1.0 M)			4.26	2.409	2.259	1.887	27	6.16	4.31	2.439	2.277	1.893
AC+ py (4.0 M)	12		4.3	2.402	2.272	1.917	28		4.32	2.37	2.28	1.92
ox-P450-camphor[a]												
high-spin		8.0				1.8						
low-spin				2.45	2.26	1.91						
ox-P450-liver microsomes[a]												
high-spin		8.1				1.7						
low-spin				2.41	2.26	1.91						

[a] references 19, 20 and 22.

for comparison. The conclusions deduced from these data are as follows : (a) The appearance of a Soret band of a complex in the absence and presence of py depended on the type of peptide, the Soret bands of the complexes containing Cys-Cys and Cys-Tyr being similar to those of P-450 enzymes. (b) All the complexes investigated showed an α-band with a shoulder, whereas P-450 has a distict α-band. (c) The EPR g-values due to formation of a ferric complex in the low-spin state in the absence and presence of py were similar to those of P-450 and model complexes retaining (S^-)-Hm-(N) coordination (1,19-21).

Blumberg and Peisach (25) and Bohan (17) used two ligand field parameters to express rhombicity and tetragonality in order to obtain information on the nature of axial coordination modes. Fig. 4 shows the ligand field correlation diagram for low-spin peptide-Hm complexes as well as heme complexes with various

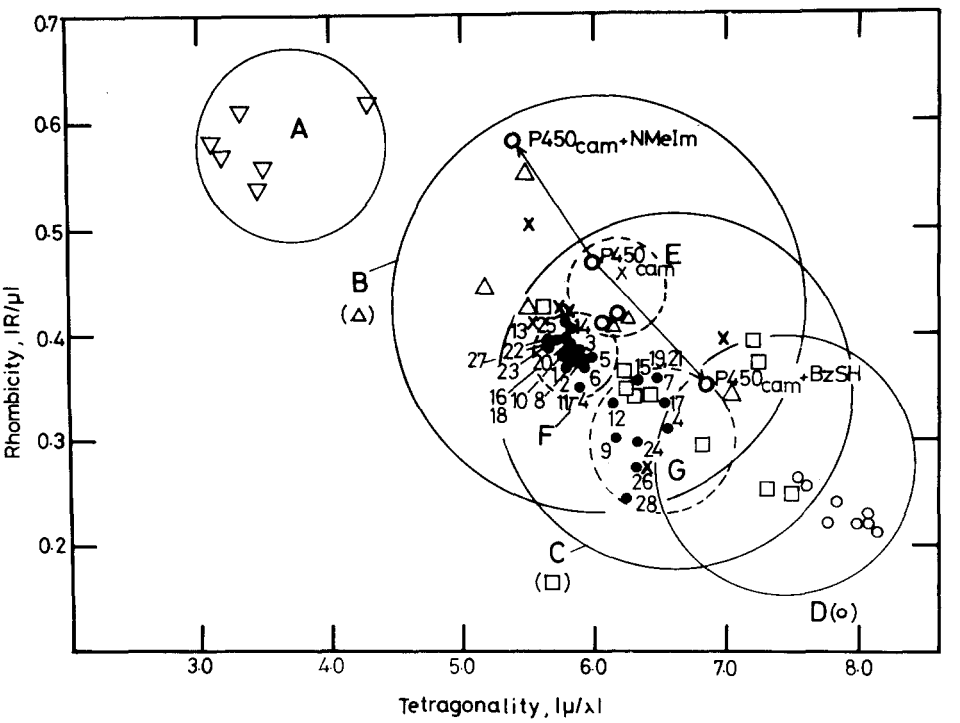

Figure 4. Correlation diagram of ligand field parameters for peptide-hemin complexes, cytochrome P-450 and various types of low-spin ferric heme complexes. Field A (▽): complex of the (N)-Hm-(N) coordination mode, Field B (△): complex of the (S^-)-Hm-(N) coordination mode, Field C (□): complex of the (S^-)-Hm-(O) coordination mode, Field D (○): complex of the (S^-)-Hm-(S^-) coordination mode, Field E (◯): cytochrome P-450's from various sources, Field F,G (●): peptide-Hm complexes. The numbers are expressed as in Table II.

axial coordination sets (13,20,21,23). P-450 enzymes are clustered in the overlapped part of the (S^-)-Hm-(N) (Field B) and (S^-)-Hm-(O) (Field C) coordination modes. As seen in Fig. 4, unlike P-450 enzymes, almost all peptide-Hm complexes prepared in the absence and presence of py were clustered in Field F near the center of Field B. Some complexes were located in a different region (Field G), suggesting the formation of an intramolecular peptide-Hm 1:1 complex including a thiolate and imidazole nitrogen coordination bond. In this work, we expected to construct the coordination site of P-450 in peptides by an intramolecular coordination to Hm through the sulfur of a Cys and oxygen of a Ser, Thr or Tyr residue. Judging from the results, this coordination seems unlikely to occur under the conditions investigated.

From our present and previously (13,26,31) findings, the 6th ligand to Hm in P-450 seems most likely to be water, which may be hydrogen bonded to protein

Table III. Hydroxylation of acetanilide by peptide-hemin complexes and rat liver microsomes[a]

system	pH	conversion of substrate(%)	product ratio(%) p-AAP : m-AAP : o-AAP
GSH-Hm	4	0.95	47 : 5 : 48
GSH-Hm	5	0.75	52 : 5 : 43
H-Hm	4	0.70	34 : 7 : 59
AH-Hm	4	0.66	33 : 7 : 60
Rat liver microsomes[a]	7.4		50 : 3 : 47

[a] reference 18.

sites (27). Our oligopeptides may help in clarifying this point, if they are suitably modified. Further work on this problem is in progress.

An interesting fearture of the model complexes is their catalytic oxidative activity on aromatic substrates, like that of P-450 enzymes. Peptide-Hm complexes such as that of H and AH catalysed the hydroxylation of acetanilide to p-, m- and o-acetaminophenol isomers in the presence of excess peptide under air. The reults are summarized in Table III with those on GSH-Hm and microsomal systems for comparison. The results indicate that sulfur-Hm bonding is essential for oxidative activity of the complexes. Studies on hydroxylation of aromatic substrates with other peptides are under way.

From the present results, thiol peptide-Hm complexes are concluded to be possible chemical models of P-450 enzymes.

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