

Models for Cytochrome P-450 under Physiological Condition

Optical properties of models for the ligation in cytochrome P-450 system were investigated by interaction of hemein with biologically significant thiol compounds such as cysteine and cysteine methyl ester, in the presence of such bases as pyridine, imidazole and histidine under physiological condition. It is shown that cysteine binds to ferric porphyrin to form axial S-Fe-N coordination in the presence of a base, and ferric porphyrin is easily reduced to ferrous porphyrin by an excess thiol. Under exposure of CO of these systems, the characteristic absorption band at 450 nm was observed, which is typical with cytochrome P-450·CO complex. These results would show that the model used nicely represent the reaction involved in the vicinity of heme moiety of cytochrome P-450 under physiological condition.

Cytochrome P-450, a protoheme-containing monooxygenases, catalyzes the hydroxylation of substrates in drug metabolism.¹⁾ Because of anomalous absorption spectrum, much interest has centered recently on the mode of axial ligand to heme iron in cytochrome P-450. The possibility of the axial cysteine-sulfur and histidine-imidazole ligations in cytochrome P-450 has been indicated in literature.²⁾

Recently, it was observed thiolates of several porphyrins exhibit similar absorption spectra and EPR parameters to those of cytochrome P-450.^{3,4,5)} Further, it was reported that the combination of reduced heme, thiol, CO and a base was able to demonstrate the Soret band at 450 nm, suggesting that a mercaptide is located at the axial coordination site of heme.^{6,7,8)}

However, these studies on model systems were carried out by use of strong bases, being different far from physiological condition, moreover, while a thiol of cysteine residue is assumed to be an axial ligand for iron in cytochrome P-450, in no case has a cysteine in the model systems been used so far.

This communication deals with the optical studies on the interactions of Hm⁹⁾ with biologically significant thiol compounds such as Cys and CysMe, and such bases as Py, Im and His under physiological condition. When Cys solution (2.0×10^{-3} — 1.6×10^{-2} M) was added to the mixture of Hm (5×10^{-5} — 10^{-4} M) in phosphate buffer of pH 7.4 at 20° in the atmosphere of air, the visible spectrum exhibited the absorption maxima at 413 nm and about 535 nm within 6 minutes in every case, which corresponds to the spectrum of typical ferric low-spin species of cytochrome P-450,¹⁰⁾ and after 5 to 20 minutes of the reaction the spectrum changed to show λ_{\max} at 415 and about 550 nm, corresponding to the spectrum of the reduced cytochrome P-450. When Im, Histm or His was used as a base, the maximum at about 510 nm was

- 1) a) V. Ullrich, *Angew. Chem.*, **11**, 701 (1972); b) I.C. Gunsalus, J.R. Meeks, J.D. Lipscomb, P. Debrunner, and E. Munk, in "Molecular Mechanisms of Oxygen Activation," ed. by O. Hayaishi, Academic Press, New York, N.Y., 1974, p. 561.
- 2) a) H.A.O. Hill, A. Roeder, and R.J.P. Williams, in "Structure and Bonding" (Berlin), **8**, 123 (1970); b) R. Tsai, C.A. Yu, I.C. Gunsalus, J. Peisach, H. Blumberg, W.H. Orme-Johnson, and H. Beinert, *Proc. Nat. Acad. Sci. USA*, **60**, 1157 (1970).
- 3) J.P. Collman, T.N. Sorrell, and B.M. Hoffman, *J. Am. Chem. Soc.*, **97**, 913 (1975).
- 4) S. Koch, S.C. Tang, R.H. Holm, R.B. Frankel, and J.A. Ibers, *J. Am. Chem. Soc.*, **97**, 916 (1975).
- 5) H. Ogoshi, H. Sugimoto, and Z. Yoshida, *Tetrahedron. Letters*, **1975**, 2289.
- 6) J.O. Stern and J. Peisach, *J. Biol. Chem.*, **249**, 7495 (1974).
- 7) a) J.P. Collman and T.N. Sorrell, *J. Am. Chem. Soc.*, **97**, 4133 (1975); b) J.P. Collman, T.N. Sorrell, J.H. Dawson, J.R. Trudell, E. Bunnenberg, and C. Djerassi, *Proc. Nat. Acad. Sci. USA*, **73**, 6 (1976).
- 8) C.K. Chang and D. Dolphin, *J. Am. Chem. Soc.*, **97**, 5948 (1975).
- 9) Abbreviation used in this work include: Hm, hemein chloride; Cys, cysteine; CysMe, cysteine methyl ester; ME, mercaptoethanol; Py, pyridine; Im, imidazole; His, histidine; Histm, histamine.
- 10) C.R.E. Jefcoate and J.I. Gaylor, *Biochemistry*, **8**, 3464 (1969).

observed in the earlier stage of the reaction (within 3 minutes), which would correspond to the high-spin state of ferric cytochrome P-450.¹⁰⁾ (Table I). However, when α -picoline was used as a base, the characteristic peaks were not induced, probably due to the steric hindrance of methyl group to ligate the heme iron.

TABLE I. Absorption Maxima^{a)} of Model Systems and Cytochrome P-450

System	pH	Reaction Time (min)	λ_{\max} (nm)		
Hm + Cys + Py	7.4	1.5		535	
		5.0	524		552
Hm + Cys + Im	7.4	1.5	510		
		4.0		539	
		20.0			555 575
Hm + Cys + Histm	7.4	3.0	510		
		5.5		537	
		11.0			550 570
Hm + Cys + His	7.4	1.0	510		
		3.0		535	
		10.0			550 570
Cytochrome P-450 ^{b)}					
{ Oxidized high-spin			505		605
low-spin				535	565
{ Reduced					555
Hm + Cys + Py + CO	6	10 → 35		455 → 450 ^{c)}	
	7	10 → 30		451 → 442	
Hm + CysMe + Py + CO	7	10 → 30		450 → 448	
Hm + ME + Im + CO	6	15 → 35		460 → 450	
	7	11 → 30		456 → 450	
	9	11 → 20		453 → 448	
	12	11 → 25		455 → 450	
Hm + ME + His + CO	9	10 → 25		450 → 445	

a) difference spectrum in CO complex

b) reference 10

c) λ_{\max} varies from 455 to 450 nm, depending on reaction time from 10 to 35 minutes after addition of thiol

From these results, it could be postulated that Cys binds to ferric porphyrin to form axial S-Fe-N coordination in the presence of a base, and within 5 to 20 minutes of the reaction ferric porphyrin is easily reduced to ferrous porphyrin by an excess thiol. To corroborate these results, EPR studies are now in progress.

When Cys ($2.5 \times 10^{-2}M$) was added to the mixture of Hm ($5 \times 10^{-5}M$) and Py ($2.5 \times 10^{-3}M$) under CO atmosphere, the absorption maximum of the solution was observed at 450 nm (10 minutes), using the mixture of Cys, Hm and Py as reference. The spectrum is typical with cytochrome P-450·CO complex.^{2,10)} As shown in Table I, the appearances of the peak at 450 nm was dependent on the physical and chemical conditions of the reaction system such as, kinds and concentration of thiol compounds and bases, the pH values of the solutions, and the reaction times after the addition of thiols. The combination of Cys, CysMe or ME with Py, Im or His, seems to be essential for bringing about the absorption band at 450 nm.

From above results, it would be concluded that (1) the thiol group coordinates as a mercaptide with heme iron in the presence of such base as Py, Im or His, (2) under exposure of CO, the base is substituted with CO.

In view of the fact that essentially the same spectral properties as in cytochrome P-450 systems were observed, the model systems used in this study would nicely represent the reaction involved in the vicinity of heme moiety of cytochrome P-450 under physiological condition.

Further, it is noteworthy that these Hm-thiol model systems demonstrated monooxygenase activity on hydroxylation of aniline and *p*-toluidine.¹¹⁾

It is highly possible that the axial ligands in cytochrome P-450 are a mercaptide of cysteine and an imidazole of histidine.

Faculty of Pharmaceutical Sciences,
University of Tokushima
Tokushima, 770, Japan

HIROMU SAKURAI

Received April 30, 1976

- 11) a) H. Sakurai and S. Ogawa, *Biochem. Pharmacol.*, **24**, 1274 (1975); b) H. Sakurai and M. Kito, *Biochem. Pharmacol.*, **24**, 1647 (1975).

[Chem. Pharm. Bull.]
24(7)1688-1689(1976)

UDC 547.918.02 : 581.192

The Structure of Cassialoin, a New Anthrone C-Glycoside from the Heartwood of *Cassia garrettiana* CRAIB.

A new anthrone C-glycoside, cassialoin was isolated from the heartwood of *Cassia garrettiana* CRAIB. (Leguminosae), a Thai drug "Sa mae sarn" being used as mild cathartics, and was elucidated as 10-hydroxy-10-C-D-glucosylchrysophanol-9-anthrone.

From the heartwood of *Cassia garrettiana* CRAIB. (Leguminosae), a Thai drug "Sa mae sarn" which is used as mild cathartics,¹⁾ in addition to chrysophanol, chrysophanol-dianthrone and (–)-11-desoxyaloin as well as some dozen kinds of phenolic substances, a new anthrone C-glycoside named cassialoin was isolated. This paper is concerned with the structure elucidation of this compound.

The methanolic extract of the plant material was chromatographed over silica gel using a mixture of hexane and ethyl acetate as the solvent. The last part of the fractions upon rechromatography over polyamide powder by elution with methanol gave pale yellow crystals of cassialoin (I), C₂₁H₂₂O₉, mp 188–191° (decomp.), $[\alpha]_D^{25} -7.6^\circ$ (*c*=1.0, ethanol). I is soluble in 5% aqueous sodium hydroxide, resulting in yellow solution which exhibits bright yellow fluorescence under ultraviolet (UV) light.

The UV spectrum of I is very similar to that of aloin and the proton magnetic resonance (PMR) spectrum gives signals due to protons of one toluene methyl group (δ 2.43), protons of sugar moiety (δ 2.8–5.7), five aromatic protons (δ 6.8–7.8) and three hydroxyl protons (δ 6.82, 11.84 and 11.94).

Acetylation of I with acetic anhydride and pyridine at room temperature gave a hexacetate (II), C₃₃H₃₄O₁₄, mp 224–226°, whose infrared (IR) and PMR spectra indicate the presence of a hydroxyl group, and II was peracetylated by the treatment with acetic anhydride and sulfuric acid, giving a heptacetate (III), C₃₅H₃₆O₁₆, mp 224–225° (decomp.). In the PMR spectra of II and III the signals due to four and five alcoholic O-acetyl groups, respectively, are observed. Therefore, I was revealed to contain an alcoholic hydroxyl group, probably tertiary, besides those of the sugar moiety.

1) Phol Phaet-Thanesuara (ed.), "Pramual Sapphakhun Ya Thai" ("Medicinal Uses of Thai Drugs"), Pt. III (Vol. 3), Samakhon Rongrien Phaet Phaen Boran (Association of the School of Oldstyle Medicine), Bangkok, 1969, p. 194.