A Chemical Model of Cytochrome P-450 with Electron-transfer Activity

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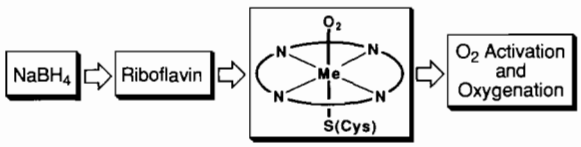
Cytochrome P-450 is a ubiquitous heme-containing enzyme that catalyzes oxidative metabolism of many chemical compounds [1]. Cytochrome P-450, which is a terminal enzyme of the electron-transfer system, is activated by NADPH-dependent cytochrome P-450 reductase as a flavin enzyme of liver microsomes in the presence of NADPH† and molecular dioxygen [1]. Recent analysis of the crystal structure of cytochrome P-450cam, which is considered a model of liver P-450, indicates that the axial donor atom *trans* to the dioxygen binding site is a negatively charged cysteinato sulfur [2]. Thus the heme–sulfur configuration is responsible for not only the formation of the reactive oxygen intermediate [3] but for the occurrence of unusual spectroscopic properties [4]. Simple chemical systems modelling the reactive center of cytochrome P-450 have been proposed in an attempt to understand the mechanism of dioxygen activation by the enzyme [5]. However, few model systems have been reported in which substrate oxygenation is facilitated by thiolato–metalloporphyrin complexes in the presence of dioxygen [6]. Furthermore, few model systems linked with a suitable electron-transfer system in the presence of a reducing agent are known [6, 7]. An interesting result has been obtained recently for a system using methylbiologen as an electron-transfer agent in the presence of an iron–porphyrin complex, zinc amalgam and acetic anhydride in acetonitrile [7].

In this communication, we describe a new system of reductive dioxygen activation at thiolato–metalloporphyrin complexes by $NaBH_4$ as a reducing agent in the presence of flavin derivative as an electron-transfer agent in benzene as a solvent (Scheme 1).



Scheme 1.

In a typical experiment, 7 μmol of metalloporphyrin, 0.57 mmol of L-cysteine·HCl·H_2O, 0.15 mmol of RTA or RTB, 1.71 mmol of KOH (dissolved in methanol) and 19.8 mmol of cyclohexene as a substrate were dissolved in benzene (total 10 ml) and 2.6 mmol of $NaBH_4$ was added to start the reaction with stirring under a 100% dioxygen atmosphere at 20 °C. The time courses of the reactions were monitored for 24 h by a GLC method.

In the complete system [$NaBH_4$/RTA/Mn(TPP)Cl/L-Cys] (Table 1, No. 1), which brought about the maximum oxygenation activity among the combinations of four components, five oxygenated products (**1**–**5**) (Scheme 2) of cyclohexene were formed, depending on the reaction time. When there was lack of RTA (No. 2), a noticeable decrease of substrate oxygenation was observed, indicating that RTA plays an important role as an electron-transfer agent. Addition of MeIm to the complete system led to a marked decrease of the substrate oxygenation (27% of the complete system after 24 h of reaction), suggesting that MeIm binds to the dioxygen binding site as the axial position of the Mn(TPP) complex. On addition of KOH to the complete system, the total oxygenation yield decreased (No. 3), however, the relative ratio of both cyclohexene oxide and cyclohexenol formed significantly increased, compared with that of the complete system (No. 1). These results indicate that proton dissociation of the thiol group of cysteine in the presence of KOH, followed by the formation of a thiolato–Mn(TPP) coordination bond is essential for dioxygen activation as well as the oxygenation of the substrate, similar to the reactions by biological systems (Table 1, No. 6–8). The use of a more lipophilic flavin such as RTB in place of RTA, resulted in improvement of the total oxygenation yield (No. 4), compared with the result of the system No. 3. Furthermore, the Fe(TPP)Cl-containing system (No. 5) was found to be a better system under the conditions tested, in terms of total oxygenation yield as well as the occurrence of a monooxygenation reaction; the main oxygenated products thereby are cyclohexene oxide and cyclohexenol. In this model system (No. 5), the formation of a ferric heme complex in the low-spin state as an intermediate complex was detectable by ESR spectroscopy measured at 77 K, probably due to the formation of a cysteinato–Fe(TPP) complex, judging from the *g* values at 2.34,

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†Abbreviations: NADPH = β-nicotinamide adenine dinucleotide phosphate, reduced form; TPP = tetraphenylporphyrin; RTA = riboflavin tetraacetate; RTB = riboflavin tetra-n-butyrate; Cys = L-cysteine·HCl·H_2O; CHP = cumene hydroperoxide; MeIm = 1-methylimidazole; $P\text{-}450_{LM}$ = cytochrome P-450 from rat liver microsome.

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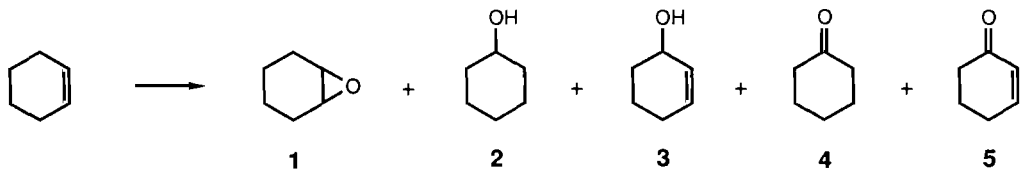
TABLE 1. Cyclohexene oxygenation by cytochrome P-450 models[a]

No.	System	Reaction time (h)	Total yields (%) based on metallo-porphyrin	Relative yields (%) of product				
				1	2	3	4	5
1	$NaBH_4$/RTA/Mn(TPP)Cl/Cys	1	7646	4	9	19	8	61
		24	33837	3	46	15	8	29
2	$NaBH_4$/Mn(TPP)/Cys	1	4130	0	0	15	7	78
		24	10304	3	20	30	6	42
3	$NaBH_4$/RTA/Mn(TPP)Cl/Cys/KOH	1	2496	13	21	67	0	0
		24	13787	4	25	42	0	28
4	$NaBH_4$/RTB/Mn(TPP)Cl/Cys/KOH	1	4490	16	11	52	t	21
		24	31322	3	13	38	1	44
5	$NaBH_4$/RTB/Fe(TPP)Cl/Cys/KOH	1	5975	13	7	80	0	0
		24	30162	4	8	51	1	36
6	Rat liver microsomes[b]	1/6		46.4	0	53.3	0	0.3
7	$P\text{-}450_{LM}$/NADPH[c]		4.2[d]	38	0	62	0	0
8	$P\text{-}450_{LM}$/CHP[c]		21.2[d]	47	0	53	0	0

[a]Data are the mean values of three experiments. [b]Data from ref. 10. [c]Data from ref. 4. [d]Turnover number per min.



Scheme 2.

2.26 and 1.95 [8]. The generation of free superoxide was not detected in the system. The oxygen-bound metalloporphyrin, Fe(IV) = O [1(g) 7], which is an ESR-silent form, is thus proposed as a possible reactive oxygen intermediate in the system.

Although several P-450 models using flavin derivatives have been reported previously [5(g), 9], the $NaBH_4$/riboflavin/metalloporphyrin/Cys/KOH system described here is the first model which has a sulfur–metalloporphyrin coordination site linked with electron-transfer activity in the presence of a reducing agent. Cytochrome P-450, which is the terminal component of the electron-transfer system, is activated by either NADPH-dependent cytochrome P-450 reductase in liver microsomes or NADH-dependent putidaredoxin reductase–putidaredoxin in *Pseudomonas putida* cytochrome P-450cam in the presence of NAD(P)H and molecular oxygen [1]. On the basis of the present experimental results, the P-450 model systems consisting of $NaBH_4$, riboflavin, metalloporphyrin, L-cysteine and KOH showing high monooxygenation activities, are proposed to have an efficient electron-transfer system, as depicted in Scheme 1, similar to the reactions of the cytochrome P-450 monoxygenase system. We are studying the mechanism of oxygenation of the system and the structure of the active oxygen intermediates.

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