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REDUCTIVE DIOXYGEN ACTIVATION BY USE OF ARTIFICIAL P-450 SYSTEMS

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CONTENTS

A. INTRODUCTION

Cytochrome P-450 was discovered in 1962 by T. Omura and R. Sato but not as "monooxygenase". The enzyme happened to be named (incorrectly) as "cytochrome" based on the similarity in its electronic spectra and magnetic property to those of the known cytochrome *b*. The name P-450 was given after the unique electronic absorption of the CO complex at 450 nm was discovered. The cytochrome P-450 family [1] is widely distributed in the animal (including human beings), plant and microbial kingdoms and participates as a monooxygenese in various detoxification [2] and biosynthetic [3] pathways which are particularly important for regulation [3] of hormone activity. Monooxygenase is the general name for the enzymes which use molecular oxygen, O_2, as an oxidant and yet introduce one

† Professor Iwao Tabushi died before he could complete the revision of the manuscript.

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oxygen atom to their substrates. Usually O_2 is cleaved "reductively" and the other oxygen atom is converted to H_2O. Biochemists are very interested in the unique mechanism [4] of the "reductive" dioxygen activation, where electrons are brought through an electron transfer system such as NAD(P)H and NAD(P)H dependent P-450 reductase [5], since this unique type of oxidation has never been found in the chemistry field. However, the detailed mechanism has been difficult for biochemists to elucidate directly by using the complex natural enzyme system in which many unusual intermediates are produced as very short-lived species. The mechanism has also attracted the attention of physical, organic and inorganic chemists who introduced the very valuable idea of the "artificial oxidant" and later the "artificial catalytic site".

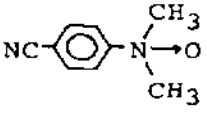
The idea of the "artificial oxidant" was first introduced by Ullrich and co-workers [6] who used iodosyl benzene (PhIO), **1**, as a possible mono-oxygen transfer reagent for cytochrome P-450. The greatest advantage in using iodosyl benzene is the fact that iodosyl benzene and iodobenzene obtained therefrom are very poor substrates, i.e. they are not easily oxygenated. Therefore, any reactive intermediate (X) once formed via O atom transfer is not destroyed easily, leaving "X" unaffected for a reasonable period of time. This further leads to the very interesting possibility that the structure of the very unstable intermediate "X" may be elucidated spectroscopically. As will be discussed later, spectroscopic data have accumulated sufficiently to indicate that the unstable intermediate "X" is a metal(V) oxene as a formal expression or metal(IV) oxene porphyrinato cation radical after internal electron transfer (see Section B on the most probable structure of the active intermediate).

Iodosyl benzene has an oxygen atom which is already activated as shown by the small bond dissociation energy of the I–O bond of 53 kcal mol^{-1}, which is much smaller than the O=O bond dissociation energy of 118 kcal mol^{-1}. The same is true for all other O atom transfer reagents listed in Table 1, whose bond dissociation energies range between ca. 30 and ca. 60 kcal mol^{-1}.

The active site of the P-450 enzyme consists of metalloporphyrin, O_2 (or corresponding oxygen atom transfer reagent such as PhIO or H_2O_2) and substrate. However, as will be mentioned later, one of the most important and characteristic facts in the P-450 catalyzed reaction is that the "electron flow" from the outer world to this active site is unusually fast. In order to achieve this unusually fast electron flow, nature probably keeps an efficient electron channel, strongly or loosely interacting with the enzyme. One of the major purposes of this review is to reveal the key role of this mysterious electron channel. The concept may also be extended to "chemi-electronics". Also recommended to readers is the excellent review of Dawson and Eble

TABLE 1

List of oxygen atom transfer reagents

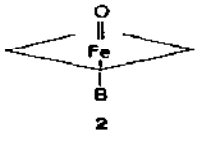
O atom transfer reagents	Bond energy (kcal mol^{-1})	Metalloporphyrin	Substrate	Ref.
PhIO	53 [a]	Fe(III), Mn(III), Cr(III)	Olefin epoxidation, aliphatic hydroxylation	7a,23a, 69c
NaOCl	52 [b]	Mn(III)	Alcohol oxidation, C–C oxidative cleavage, other oxidation scission	22
NaOCl LiOCl	52 [b]	Mn(III)	Olefin epoxidation Aliphatic hydroxylation	27 24
	61 [c]	Fe(III), Mn(III), Cr(III)	Olefin oxidation, aliphatic hydroxylation, t-amine dealkylation	28
ROOH H_2O_2	53–36 [d] 52 [e]	Fe(III), Mn(III)	Olefin epoxidation, aliphatic hydroxylation	29

[a] I–O bond energy, ref. 71.
[b] Cl–O bond energy in the Cl oxide, ref. 72.
[c] N → O bond energy in dimethyl buroxan, ref. 73.
[d] R = alkyl and acyl, ref. 74.
[e] Ref. 75.

[4c] which is written from a quite different point of view from the present one; it may be useful to read both.

B. PROPERTIES AND STRUCTURE OF THE P-450 ACTIVE INTERMEDIATE

As the most plausible structure of the active intermediate of the natural cytochrome P-450 cycle, porphyrinato iron(V) oxene, **2**, was first suggested



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by Groves et al. based on substrate oxygenation [7a] and spectroscopic data [7b,c]. The iron(V) oxene structure (or porphyrinato iron(IV) oxene cation radical after internal electron transfer) was ultimately proven for the related natural enzyme, horseradish peroxidase (HRP) when treated with H_2O_2, by use of EXAFS [8], Mössbauer spectroscopy [9], resonance Raman spec-

TABLE 2

Spectroscopic data for porphyrinato metal oxene and compounds related to P-450 intermediates

Type of compound	Preparation conditions	Spectroscopic results	Ref.
P·Fe(II)·O_2	T_{piv}PP·Fe(II)·NMeIm + O_2	Electron absorbance at 429, 548 nm Mössbauer δ 0.29 0.28 0.25 ΔE_Q 2.10 2.00 1.34 Magnetic susceptibility $S = 0$	30
P·Fe(II)·O_2^-	TPP·Fe(II) + KO_2 OEP·Fe(II) rT, in DMSO	ESR (at 77 K) $g \cong 4.2$, 2 (high spin Fe(III)) Electronic absorption 437, 547, 565, 597, 609 nm (for TPP) 427, 527, 545, 573, 584 nm (for OEP)	18(a)
	TPP·Fe(II)·O_2 OEP·Fe(II)·O_2 (electrochemical) −25 °C in CH_3CN–DMSO	Same results as above	18(b)
P·Fe(V)O	TMP·Fe(III) + PhIO or *m*-CPBA −78 °C, in CH_2Cl_2–CH_3OH	Electron absorbance at 406, 645 nm ESR silent EXAFS Fe–O 1.6 Å Mössbauer, $\delta = 0.05$ mm s^{-1} $\Delta E_Q = 1.49$ mm s^{-1} Magnetic susceptibility $S = 3/2$* ($S = 1$ for Fe(IV) and and $S = 1/2$ for Por^+) ^{1}H NMR, Curie law, paramagnetic	7(b)
P·Mn(II)·O_2	TPP·Mn(II) + O_2 −79 °C in toluene	Electronic absorbance at 472, 542, 638, 820 nm ESR $g = 1.45$, 1.995 5.424, 5.470 ($A = 88$ G)($A = 57$ G) ($Mn^{IV}O_2^{2-}$, $S = 3/2$)	31
P·Mn(V)·O	TMP·Mn(III) + KO_2 + *m*-ClPhCOCl + OH^-, −30 °C, in CH_2Cl_2	Electronic absorbance at 422 nm ^{1}H NMR δ, 10.5 (mH) at −31 °C 12 (mH) at −68 °C	23(a)
	TMP·Mn(III) + NaOCl(NaOBr) r.t. in CH_2Cl_2/H_2O P·Mn(V)·O formed in CH_2Cl_2 was immediately transferred to cold (−80 °C) hexane	Electronic absorbance at 425 nm IR 1260, 965, 710, 605 cm ^{1}H NMR δ, 10.1, 11.0(mH) at −30 °C 10.6, 11.7(mH) at −60 °C 11.6, 12.5(mH) at −80 °C Magnetic susceptibility $S = 3/2$ (Antiferromagnetic coupling of Mn^{IV} ($S = 3/2$) and P_{or}^+ ($S = 1/2$))	26

troscopy [10], ^{1}H NMR [11], etc. Mössbauer measurements of compound I derived from HRP with H_2O_2 in solution (at 4.2 K) gave $\delta = 0.08$ mm s^{-1} and $\Delta E_Q = 1.25$ mm s^{-1}, strongly suggesting that the cation radical of the porphyrinato Fe(IV) oxene is the most dominant limiting structure [9]. Observed total spin, estimated from its magnetic susceptibility, is close to unity ($S = 1$), also supporting the Fe(IV) structure. The corresponding model system, tetramesitylporphyrin (TMP) Fe oxene, showed very similar Mössbauer data, $\delta = 0.05$ and $\Delta E_Q = 1.49$ [7b]. The EXAFS measurements of the TMP Fe oxene showed an Fe–O distance of 1.6 Å (double bond), supporting the oxene structure. Other circumstantial evidence supports this conclusion. For the corresponding Mn complex, a similar conclusion was drawn. The same intermediate is also formed for cytochrome *c* peroxidase [12] when treated with H_2O_2. Other spectroscopic data, as well as the physical properties of porphyrinato iron(V) oxene are listed in Table 2. The real structure, of course, would be described by configuration interaction of several possible limiting formulae (P · M(V)=O, $^{+}$P · M(IV)=O, $^{+}_{+}$P · M(III)=O, etc.) and the contribution and the structure of the most predominant species may change depending on the microenvironment. The structure of cytochrome P-450 is described as P · M(V)=O hereafter, just for simplicity.

C. MONOOXYGENATION OF VARIOUS SUBSTRATES WITH PORPHYRINATO METALLO(V) OXENES PREPARED FROM OXYGEN ATOM TRANSFER REAGENTS

Natural P-450 enzyme (P-450 LM [13] or P-450 cam [14]) when treated with an oxygen atom transfer reagent such as H_2O_2 [15], periodate [16], or organic peracids [17] such as *m*-CPBA or PhIO [6,33] in the presence of a large excess of a suitable substrate, gave the corresponding monooxygenated or oxidation products. These reactions proceed in catalytic fashion in most cases. Similarly, the corresponding artificial porphyrinato Fe(III) or Mn(III) catalyzes the monooxygenation and the related oxidation reactions. Results are summarized in Table 3. When H_2O_2 is used, the reaction follows the so called "shunt pathway" [36] (see Fig. 1) in which porphyrinato metal hydroperoxide may be formed as a key intermediate. The structure was characterized for TPP · Fe^{III} · O_2^{2-} and OEP · Fe^{III} · O_2^{2-} based on ESR, electronic absorption, IR and Raman spectra [18]. The hydroperoxy complex **3** is also considered as the key intermediate in the reductive O_2

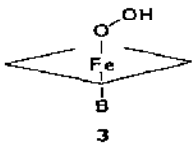

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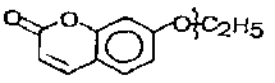
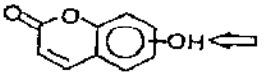
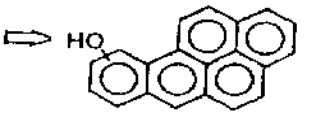
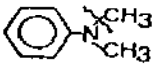
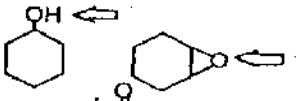
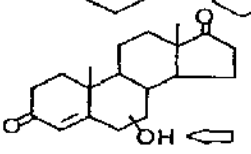
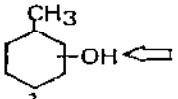
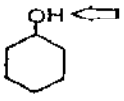
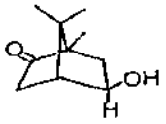
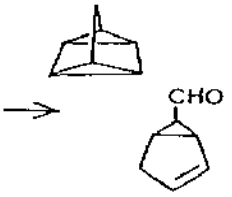
TABLE 3

Substrate oxygenation or oxidation with oxygen atom transfer reagents in the presence of the natural P-450 enzyme

O atom transfer reagents	Natural enzyme system	Substrate oxygenation	Reference
PhIO	Liver microsome		6
Cumene hydroperoxide H_2O	Liver microsome	 	15
Cumene hydroperoxide H_2O_2, peracids	P-450 LM	 	13
$NaIO_4$, $NaClO_2$	Liver microsome	 6β, 7α, 16α position was hydroxylated	16
p-$CH_3C_6H_4SO_3N{=}IC_6H_5$	P-450 LM_2	 $^{1,3}cis$,^{4}cis,$^{3}trans$· $^{4}trans$ mainly ^{3}cis 	32
PhIO Cumene hydroperoxide m-CPBA	P-450		34
PhIO	Liver microsomes		35

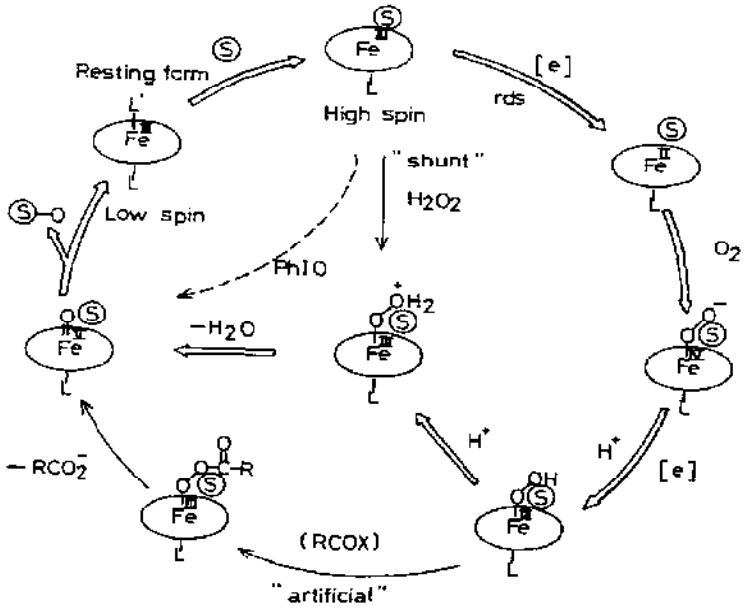


Fig. 1. P-450 catalytic cycle.

activation based on aliphatic hydroxylation assisted by acid anhydride [19]; the kinetic analysis of its formation will be discussed in detail later.

Porphyrinato–Mn(III) was readily oxidized with OCl^- to give the corresponding Mn(IV) as the stable product [20].

$$2P \cdot Mn(III) + OCl^- + 2H^+ \rightarrow 2P \cdot Mn(IV) + H_2O + Cl^- \quad (1)$$

The oxidation proceeds smoothly in homogeneous solution but the resultant porphyrinato–Mn(IV) is too unstable to exist in an aqueous solution for a reasonable period of time except in strongly alkaline conditions. As a result, the P · Mn(III) + NaOCl system is not an appropriate oxidant for ordinary substrates. The problem was studied in much more detail by us [21a,b], firstly through the dependence of the oxidation potential of the porphyrinato–Mn(IV) complex on the pH of the aqueous solution [21a]. The Mn(IV) complex oxidizes a water molecule below pH 9.9, to produce H_2O_2 and a small amount of O_2 [21a]

$$P \cdot Mn(IV) + H_2O \xrightarrow{pH < 9.9} P \cdot Mn(III) + H^+ + \tfrac{1}{2}H_2O_2 \quad (2a)$$

$$H_2O_2 \xrightarrow{\text{Mn complex}} H_2O + \tfrac{1}{2}O_2 \quad (2b)$$

Since the very strong oxidant P · Mn(IV) or its related species is formed in reaction (1), organic compounds having oxidation potentials smaller than or similar to that of water were investigated. As shown in Fig. 2, some

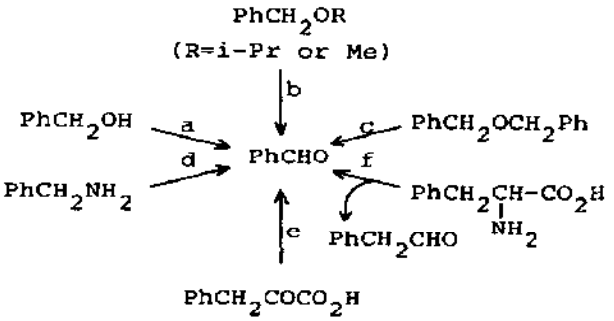


Fig. 2. Organic oxidation reactions carried out with "high potential Mn".

interesting oxidation reactions were observed for certain organic substrates [22a].

When a hydrophobic porphyrinato–Mn(III) was used as the reaction center, it was better to use an appropriate organic phase. In the two phase system, the oxidation of Mn(III) complex with OCl^- becomes even slower, limiting the applicability of the system to catalytic oxidations, though the active intermediate is strong enough to oxidize most organic substrates. This extreme slowness of conversion from Mn(III) to Mn(IV) or related species was dramatically improved when quaternary ammonium $(C_8H_{17})_3NMe^+$, **4**, was used under the phase transfer conditions [22b]. A rate enhancement by a factor of 260 was observed for NaOCl + TOMA · Cl + TPP · Mn(III) · Cl (see Table 4) [22b]. This remarkable enhancement showed the phase transfer system, $P \cdot Mn(III)_{org} + NaOCl_{aq} + R_4N^+$, to be one of the most efficient catalytic oxidation systems, since the elementary reaction between a substrate(s) and P · Mn(IV) or related active species usually proceeds quite rapidly.

Later, based on indirect evidence, the formation of Mn(V)=O was proposed as an active species [23a,b]. This hypothesis explains the situation clearly, since the disproportionation between Mn(V) and Mn(III) is known

TABLE 4

Rate constants of phase transfer and electron transfer catalysis

Oxidant	Δ(PhCHO)/Δt($\times 10^3$) (min^{-1} M)	Rate constants
NaOCl	1.5	0.0083 min^{-1} M^{-1}
NaOCl + TOMA · Cl	4.5	0.930 min^{-1} M^{-2}
NaOCl + TPP · Mn(III) · Cl	4.4	1.460 min^{-1} M^{-2}
NaOCl + TOMA · Cl + TPP · Mn(III) · Cl	65	375 min^{-1} M^{-3}

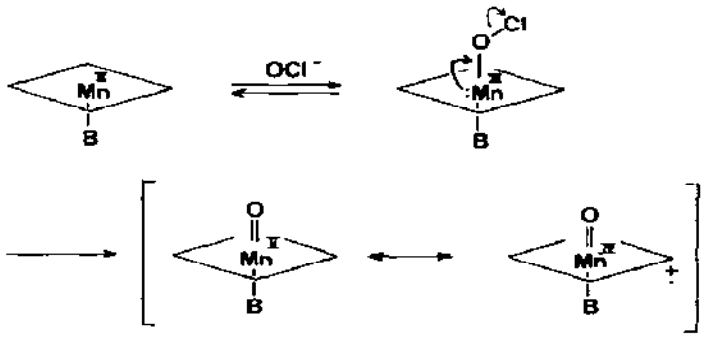


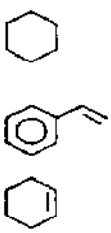
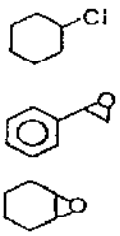
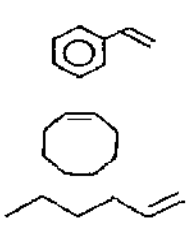
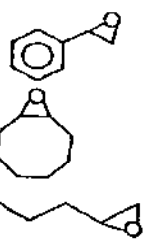
Fig. 3. Mn oxene formation using OCl^- as an O atom transfer reagent.

to proceed very rapidly to give Mn(IV) as a more stable product [23b]. The proposed reaction is shown in Fig. 3. Therefore, the very rapid and efficient oxidations found by Tabushi and Koga [22] most probably involve the P-450 active intermediate shown in Fig. 3.

A small but interesting modification was reported by Meunier and co-workers [24,25] who used pyridine in Tabushi and Koga's phase-transfer system. This ligand stabilized the metal oxene intermediate to keep the

TABLE 5

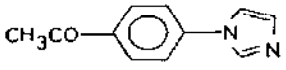
Organic oxidation with a P·Mn(III) + NaOCl system

System	Solvent	Substrate	Product	Ref.
TPP·Mn(III)·Cl NaOCl $(n\text{-octyl})_3N^+Me$	CH_2Cl_2/ H_2O	$PhCH_2OH$ $(PhCH_2)_2O$ $PhCH_2COCO_2H$	PhCHO PhCHO PhCHO	22
TPP·Mn(III)·OAc NaOCl Pyridine [a] $CH_3(CH_2)_{13}N(CH_3)_2Ph$	CH_2Cl_2/ H_2O			24
TPP·Mn(III)·Cl LiOCl NAcPhIm $CH_3(CH_2)_{13}N(CH_3)_2Ph$	CH_2Cl_2/ H_2O			27

[a] Pyridine was oxygenated to pyridine *N*-oxide.

oxene lifetime long enough for its direct spectroscopic observation [26]. Unfortunately, pyridine is also a good substrate for the metal oxenes (see Table 5) and appropriate molecular design is necessary to gain good "turnover" for the oxidation.

Appropriate molecular design may be carried out by fixing a ligand to one side of a porphyrin plane to separate it from the active oxygen of the oxene to be formed on the other side of the porphyrin plane. An alternative design, which was actually taken successfully by Collman et al. [27], is based on "attenuation" of electron density of a ligand. If a ligand is too electron rich, its oxidation becomes easy, reducing the turnover. If a ligand is too electron-poor, its coordination becomes insufficient, making the overall catalytic cycle less frequent. Through optimization Collman et al. [27] observed very good turnover frequency for epoxidation of styrene by use of **5** and NaOCl.



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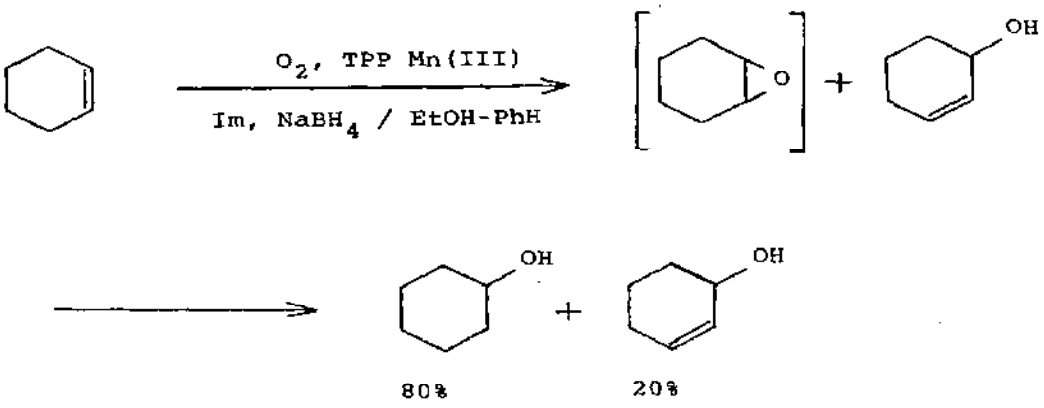
D. THE RECENT RAPID DEVELOPMENT OF THE ELUCIDATION OF THE P-450 MECHANISM

Although monooxygenation looks like a very simple reaction, e.g. smooth oxygen atom "sliding" or "insertion" from the P-450 active center to olefin, the actual mechanism involves many complications, such as H^- migration in the substrate. Especially, the nature of the reactive intermediates during the olefin [37b,41] or paraffin oxidation [38] is questionable. A particular question is whether or not a tetrahedral [27,37b,39] adduct is formed. The other extreme case is, of course, cation radical formation [40,41]. How does olefin participation determine the spin state of the P-450 enzyme [4c]? Special applications of spectroscopies under extreme conditions also give interesting information on P-450 [4c,7b,41]. Chiral induction and chiral selection of P-450 are other challenging problems which should be solved [37a,38]. In every area rapid progress has been made; among them some are still growing, and in other areas, there are contradictory mechanisms. The number of published articles which treat these problems is now increasing rapidly. Under these circumstances I only show here several examples and would like to put the collection of the rest of the important literature in the reader's hands.

(i) Reductive dioxygen activation

Although the structure of the P-450 active species has been elucidated through data accumulated for the related heme enzymes, especially horsera-



(3)

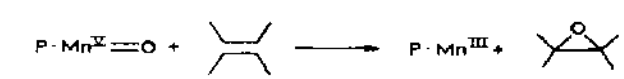
dish peroxidase, studies on the reaction mechanism of "reductive dioxygen activation" made almost no progress until 1979, when the first success in the epoxidation of olefins with O_2 in the presence of $NaBH_4$ and totally artificial porphyrinato–Mn complex, was reported [42] (eqn. (3)).

As expected, the reaction is not simple and is contaminated with free-radical chain autoxidation. The latter, a rather general problem for the slow P-450 type of reaction, was easily and satisfactorily suppressed by the addition of a free-radical quencher such as 2,6-di-tert-butyl-*p*-cresol [42]. An interesting observation after the "isolation" of the artificial P-450 type reaction is the fact that the chemoselectivity between allylic oxidation and epoxidation for the artificial P-450 type reaction is entirely different from the chemoselectivity for the autoxidation (see Table 6), almost discriminating epoxidation for the P-450 type while almost exclusive allylic oxidation for the autoxidation. This remarkable change in the chemoselectivity may also be true for O atom transfer reactions, from most of which trace O_2 has not been rigorously excluded. Therefore, some of the chemoselectivities reported in the literature (often too favorable for allylic oxidation) may be overall values including autoxidation.

The use of BH_4^- as an efficient electron donor successfully opened a new gate to reductive O_2 activation and showed that the key step was the reduction of porphyrinato–Mn(III) to the Mn(II) complex. The following steps are incredibly simple, proceeding spontaneously as

$$P \cdot Mn^{III} \xrightarrow{e^-(BH_4^-)} P \cdot Mn^{II} \xrightarrow{O_2} P \cdot Mn^{II} \cdot O_2 \xrightarrow{H^+(EtOH)} P \cdot Mn^{IV} \cdot O_2H$$

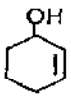
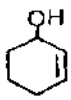
$$\xrightarrow{e^-(BH_4^-)} \xrightarrow{H^+(EtOH)} P \cdot Mn^{V}{=}O \qquad (4)$$



(5)

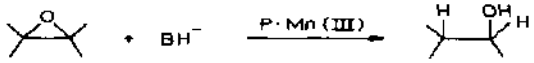
TABLE 6

Comparison of chemoselectivity between P-450 type reaction and autoxidation

System	Epoxidation (%)	Allylic oxidation (%)		Ref.
Autoxidation TPP·Mn(III)·Cl O_2				42
	(2)	(80)	(18)	
P-450 type reaction TPP·Mn(III)·Cl O_2 $NaBH_4$				42
	(80)	(20)		
TPP·Mn(III)·Cl O_2 H_2-Pt/PVP N-MeImd				50
	(93)	(6)	(<1)	
TPP·Fe(III)·Cl PhIO				7a, 37a
	(79)	(21)		

in which two striking observations were made: (i) EtOH, a very weak proton donor, satisfactorily protonates the oxy (O_2) and hydroperoxy (O_2H) complexes, and (ii) the Mn(V) oxene, a very strong oxidant, is formed in the strongly reducing environment. These two facts were entirely unexpected from the general idea about P-450 at that time, but very encouraging at least for us to find better reductive O_2 activation systems.

Yet a system better than $NaBH_4$ was really needed, since BH_4^- was activated by porphyrinato–Mn^{III} to cleave epoxides reductively as shown in eqn. (6).



(6)

The reaction proceeded smoothly and the conversion under recycling oxygenation conditions was nearly quantitative. Another problem in using BH_4^- as the electron source is, of course, contribution of BH_3 as a real reactant (hydroboration is a typical example).

Replacement of BH_4^- by an appropriate reductant was, therefore, really important to overcome the first barrier. In our search for a better and

TABLE 7

Electron transfer rate constant from $(Pt)_x \cdot (e)_n$ to P·M(III)

Substrate	Redox potential	k ($M^{-1}\,s^{-1}$)	Ref.
TPP·Mn^{III}·Cl	−0.27 [a]	8.1×10^7	50
T_{piv}PP·Fe^{III}*N*-MeIm, Cl	−0.11 [b]	7.3×10^7	52
Cyt *c*	+0.25 [c]	1.5×10^5	53
Cyt c_3	−0.31 to −0.20 [c]	6.2×10^4	53

[a] Ref. 76.
[b] Ref. 77.
[c] Ref. 48.

"cleaner" reductant, H_2/colloidal Pt was among the best studied. Colloidal Pt was first introduced by Rampino and Nord [43] and after that discovery, many people used colloidal Pt as an efficient catalyst converting electrons to H_2, especially in the field of solar energy conversion, in which an irradiated photo reaction center ejected electrons [44]. Our own observation, however, was that colloidal Pt was an even better catalyst for the retroconversion from H_2 to electrons. Colloidal Pt is a colloidal Pt particle of ca. 30 Å diameter which is supported on a synthetic polymer of molecular weight 5×10^3–1×10^5 dalton. Supporting polymers may be polyvinylalcohol (PVA), polyvinylpyrrolidione (PVP) or polyvinylether (PVE), each of which has a strong affinity not only for colloidal Pt but also for a certain range of solvents [45]: water, methanol and ethanol for PVA [43]; aromatics, aliphatics for PVP [46], etc. Therefore, almost any solvent may be used by proper choice of the supporting polymer [45].

The high efficiency of colloidal platinum is demonstrated by the near diffusion-controlled electron transfer to porphyrinato–Mn(III) or –Fe(III) (see Table 7). In fact, the observed second-order rate constant for electron transfer, which is quite reproducible and therefore valuable for evaluation of the catalytic efficiency of some aged colloidal Pt, is even larger than the diffusion limit. This may be due to multi-site absorption equilibrium of porphyrinato–metal(III) onto the colloidal Pt surface, judging by the absorption spectral change of the porphyrinato–metal(III) complex [47].

The cleanness of the H_2/colloidal Pt (abbreviated as $(Pt)_x$)/porphyrinato–metal(III) system is best described by the simplicity of its stoichiometry shown in eqn. (7), in which production of an acid is the sole side reaction.

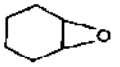
$$(Pt)_x + H_2 \rightleftarrows (Pt)_x^{2-} + 2H^+ \tag{7a}$$

$$(Pt)_x^{2-} + 2P\cdot M(III)\cdot Cl^- \rightleftarrows (Pt)_x + 2P\cdot M(II)\text{–}2Cl^- \tag{7b}$$

The side reaction is easily controlled by using a buffer system, if necessary.

TABLE 8

Reaction conditions for reductive activation of O_2 with $(Pt)_x \cdot (e)_n$

Reaction material	Solvent	Partial pressure (atm)	Temperature (°C)	Yield (%)
H_2, O_2, $(Pt)_x$ TPP·Mn(III)·Cl	benzene : EtOH (v/v, 2 : 1)	$P_{H_2} = 1/2$ $P_{O_2} = 1/2$	25 ± 1	H_2O_2 (2)
H_2, O_2, $(Pt)_x$	benzene : EtOH	$P_{H_2} = 1/2$	25 ± 1	
TPP·Mn(III)·Cl	(v/v, 2 : 1)	$P = 1/2$		(6470) [a]
N-MeImd, 				
$(Pt)_x + H_2 + O_2$ $(3.5 \times 10^{-9}$ mol)	benzene : EtOH (v/v, 2 : 1)	$H_2 = P_{O_2} = 1/2$	25 ± 1	H_2O_2 $(< 2.5 \times 10^{-7}$ mol)
H_2O_2 + P·Mn(III) + 				
$(< 2.5 \times 10^{-7}$ mol)	$(1.4 \times 10^{-5}$ mol)			$(< 1.24 \times 10^{-7}$ mol) [b] (< 0.9%) [a]

[a] Yield based on TPP·Mn(III)·Cl employed.
[b] Amount of product.

When O_2 is introduced, the situation becomes somewhat more complex but, of course, this is inevitable. The major problem may be direct electron transfer from $(Pt)_x$ to O_2 to give H_2O_2 as one of the major products [43]. Fortunately, however, the observed production of H_2O_2 for the $H_2/O_2/(Pt)_x$ system was very minute, and might be responsible for ca. 5% of the epoxide formed in the presence of tetraphenylporphyrinato–Mn(III) under the corresponding conditions described in Table 8. Superoxide, O_2^-, the other major product derived from the electron transfer does not cause any problem, since the superoxide pathway comes back to join in the main O_2 activation pathway at the stage of the hydroperoxy complex, after making a short "detour" as shown in eqn. (8) [48].

$$(Pt)_x^{2-} + O_2 \rightarrow (Pt)_x^{-} + O_2^-$$
$$O_2^- + P \cdot M(II) \rightarrow P \cdot M(III)O_2^{2-} \xrightarrow{H^+} P \cdot M(III)O_2H \qquad (8)$$

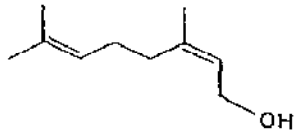
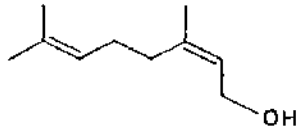
The detour mechanism, in a more restricted way and qualitative way, was originally proposed by Groves et al. [23a].

(ii) The mechanism of reductive O_2 activation through use of the H_2/colloidal Pt/porphyrinato metal(III) artificial P-450 system and problems therein

Serious mechanistic problems in the $NaBH_4$ dependent artificial P-450 system mentioned above, were completely solved by use of the H_2/colloidal

TABLE 9

Turnover number for epoxidation of olefin catalyzed by the artificial P-450 system

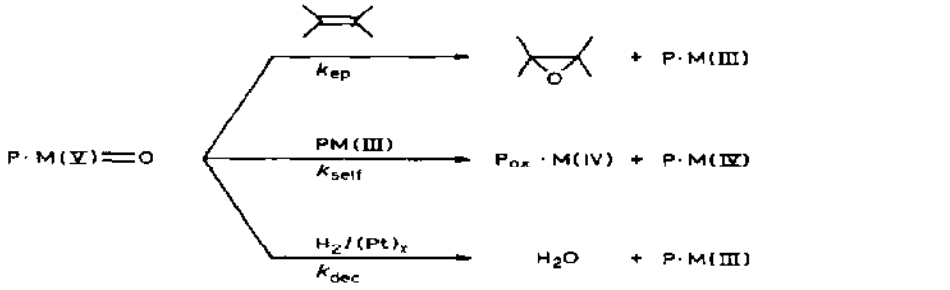
System	Substrate	Turnover number [a]	Ref.
TPP·Mn(III)·Cl $NaBH_4$, O_2		15.3	42
TPP·Mn(III)·Cl H_2-Pt/PVP, O_2 *N*-MeImd		64.7	50
TPPS·Mn(III) H_2-Pt/PVA, O_2 N-MeImd		143.9 (533.0)	51
TPPS·Mn(III) MeNAH/FMN, O_2 *N*-MeImd, $(PhCO)_2O$		260.0 (1857.1)	63
TPP·Fe(III)·Cl PhIO		9.1	7a, 37a

[a] In parentheses, turnover number based on quantity of TPPS·Mn(III) decomposed, is shown.

Pt system and the first test reaction was applied to olefins. Cyclohexene and other olefins were very cleanly epoxidized with satisfactory turnover numbers [49,50] (see Table 9), and allylic hydroxylation was a minor reaction.

(9)

The turnover numbers observed, for the epoxidation for example, (mol epoxide)/mol P · Mn(III) employed, were dependent on the reaction conditions, most significantly on the overall "reactivity" and the concentration of the olefin to be epoxidized, since the observed turnover number should be mainly controlled by the competitive reactions



(10a)

(10b)

(10c)

Therefore, the "selectivity factor" should be defined as

$$S_i = \frac{k_i[A]_i}{\sum_{i=1}^{3} k_i[A]_i} \tag{11}$$

where $[A]_i$ represents concentration of olefin, catalyst P · M and the product $[H_2] \cdot [(Pt)_x]$. Another important factor to be considered is the rate determining P · M(V)=O production. However, except for very unusual conditions, from the O_2 binding (to P · M(II)) to the oxene formation, every elementary step is quite (and unexpectedly) fast, as will be discussed later, and the overall rate determining step is the reduction of P · M(III) to P · M(II). Therefore, it is quite simple to write the simple rate equation

$$v = k_{red}[P \cdot M(III)][e] \tag{12}$$

which is not affected much by O_2 partial pressure or acidity of the solution, if these are in reasonable ranges. Based on eqns. (11) and (12), the "turnover frequency", i.e. [mol epoxide formed]/[mol P · M(III) employed] × [unit time], is given, provided an important assumption is made that the self-destruction pathway (eqn. 10b) is slow enough, by the rather simple equation

$$\text{(turnover frequency)} = k_{red} \cdot [e] \cdot S_{ep} \tag{13}$$

$$= [\text{electron influx}] \cdot S_{ep} \tag{13a}$$

An important message from eqn. (13) is that not only is the "chemoselectivity" favorable for the monooxygenation but also the absolute magnitude of the "electron influx" is incredibly important for a very stable P-450 type artificial catalyst (native enzyme, too!). This will be discussed further in a later section.

Let us pay more attention to the "chemoselectivity", S_i. If our discussion on "chemoselectivity" is oriented toward better turnover numbers, what we have to worry about is clear—selectivity between reaction (10a) and (10b). However, there are few choices! We can do something, but not much, about the enhancement of k_{ep} (this will be discussed in the final section). [P · M(III)] may be reduced, but only with a serious loss of "actual production" (mol epoxide formed per unit time) and the solution is too superficial. Thus, we now have two trump cards in our hands: possible reduction of k_{self} by appropriate molecular design and a great increase in the olefin concentration. The latter has, of course, a solubility limit and frequently there is a sort of "solution property limit" below the former limit. This problem is easily solved and the turnover number is readily optimized. Further sophisticated improvements will be described in a later section. Through this simple

optimization, the observed turnover number reached 500 (mol mol^{-1}) or even ca. 1800 for the nerol epoxidation [51]. Yet the reaction proceeded rather slowly, since the P-450 type intermediate still preferred to take pathway (10c), a useless decomposition to give the "resting" P · M(III) again.

Another interesting challenge is to stabilize or hopefully, to superstabilize porphyrin ligand against "self-oxidative destruction", (10b). The reaction is now known to be not so simple as (10b) woud suggest. Besides the oxidation of the porphyrinato metal as the wrong substrate, there may be complex reactions involving olefin addition [37b,39] followed by intramolecular *N*-alkylation [54,55] which may lead to the P-450 type product but also may lead to an irreversible side product; details are still under discussion.

One way to protect the porphyrin residue against undesirable oxidation or other related reactions, is to introduce strongly electron-withdrawing substituent(s) directly or indirectly onto the substituents already on the porphyrin ring. A typical example of the second approach is chloro- or fluorosubstitution on the phenyl rings of tetraphenylporphyrin (TPP) [56,58]. In this way, the basic (favorable) characteristics of the parent TPP are at least partly preserved. There are still several problems: (1) for any electron deficient porphyrinato metal(II) or metal(III), the O atom transfer or O_2 binding becomes less favorable; (2) preparation of a halosubstituted, or of any other (suitably) heteroatom-substituted porphyrinato metal, is always more time consuming and much more expensive than the corresponding unsubstituted compound. Problem (1) may be solved, at least for the O atom transfer by using a much stronger O transfer reagent. For example iodosyl perfluorobenzene is a very powerful O atom transfer reagent, readily transfering an O atom to the fluoro TPPM to produce the corresponding metal oxene [56]. However, any powerful O atom transfer reagent X–O has a small X–O bond dissociation energy, which may cause another, perhaps more serious problem. A couple of violent explosions have already been reported for iodosyl perfluorobenzene.

Coming back to O_2 activation, it is interesting for us to note that these "everlasting" haloporphyrins were reported not to last long in practical catalytic systems [58], as will be discussed further in Section F. The last problem in the stabilization of the porphyrins is rather general; what is the balance between how much is to be gained and how much is to be lost by the halogenation or any other modification? The basic equation is quite simple; how much product (in $, not in mols) is produced by unit time. Apparently, a small amount of a sophisticated catalyst means a lot of $. If its lifetime (turnover) is short, such sophistication, however beautiful in a written chemical equation, is a sort of "gorgeous dessert in a painting" (old Japanese saying).

Further discussion in this section, therefore, is simple and strictly oriented toward "kinetic optimization" of the simple artificial P-450 system—the simpler, the better!

Let us reconsider O_2 activation and the product determining step (10). It is obvious that rate equations and rate constants are needed, but one must notice that the rate controlling step is the production of the working $P \cdot M(II)$ from resting $P \cdot M(III)$, which usually follows the rate eqn. (11) with a rate constant to be easily measured. Then, the next question is how $P \cdot M(II)$ reaches $P \cdot M(V)=O$. According to the literature, as well as our own results, there are at least several "detour" pathways, as shown in eqns. (14) and (15).

$$\xrightleftharpoons{O_2,\ k_{O_2} \text{ and } K_{O_2}} P \cdot M(II) \cdot O_2 \tag{14a}$$

$$P \cdot M(II) \xrightleftharpoons{O_2,\ K} P \cdot M(III) + O_2^- \tag{14b}$$

$$\xrightleftharpoons{O_2^-,\ k_{SO} \text{ and } K_{SO}} P \cdot M(II) \cdot O_2^- \tag{14c}$$

$$P \cdot M(II) \cdot O_2 (\leftrightarrow P \cdot M(IV)\text{–}O\text{–}O^-) \xrightarrow{H^+,\ e^-} P \cdot M(III)O_2H \tag{15a}$$

$$P \cdot M(II) \cdot O_2^- (\leftrightarrow P \cdot M(III)\text{–}O\text{–}O^-) \xrightarrow{H^+} P \cdot M(III)O_2H \tag{15b}$$

$$P \cdot M(II) \cdot O_2 + P \cdot M(II) \xrightarrow[k_{PO}]{} P \cdot M(III)\text{–}O\text{–}O\text{–}M(III) \cdot P \xrightarrow{-O} P \cdot M(III)\text{–}O\text{–}M(III) \cdot P \tag{15c}$$

$$P \cdot M(III)O_2H \xrightarrow{H^+} P \cdot M(III)O_2H_2^+ \xrightarrow{-H_2O} P \cdot M(V)=O [\leftrightarrow {}^{\ddagger} PM(IV)=O] \tag{16}$$

Since all of these steps come after the rate determining electron transfer step (12), yet before the product determining step (10), kinetic information is hardly obtainable for steps (14) and (15). In order to get the kinetic information necessary for mechanism elucidation and catalysis optimization, we have chosen Collman's picket fence porphyrin [52a] as the best candidate for providing the stable oxy complex in a nearly quantitative fashion.

Optimization of the oxy population was successfully carried out through our equilibrium constant measurements. In this way, the oxy complex population was adjusted to 90–100% under stopped-flow kinetic conditions for study of the decay of the oxy complex (see Table 10). All of the partial pressures of O_2 and H_2, the $(Pt)_x$ concentration, the imidazole concentration (by keeping the $[Im]/[ImH^+]$ ratio constant), acidity (by keeping [Im] constant), and the concentration of porphyrinato iron were independently varied.

TABLE 10

Picket fence Fe(II) oxy complex formation under kinetic conditions

Parameter	Value
$T_{piv}PP \cdot Fe(II)$ concentration (M)	4.55×10^{-6} to 13.5×10^{-6}
Imd concentration (M)	0.182×10^{-4} to 16.2×10^{4}
O_2 partial pressure (mm Hg)	95–380
pH	6.35–7.94
$T_{piv}PP \cdot Fe(II)ImdO_2$ yield at 25.0 °C (%)	90–100

A couple of examples are shown in Figs. 4 and 5. From a series of rate measurements, a rate eqn. (17) was obtained.

$$\frac{-[\mathrm{d\ P \cdot Fe(II) \cdot Im \cdot O_2}]}{\mathrm{d}t} = k[\mathrm{P \cdot Fe(II) \cdot Im \cdot O_2}][(\mathrm{Pt})_x][\mathrm{H_2}](\alpha + \beta h) \tag{17}$$

where Im denotes imidazole or *N*-MeImd, $[H_2]$ partial pressure of hydrogen, h Hammett's constant similar to proton concentration, and α and β are constants experimentally obtained from Fig. 5.

Some interesting aspects are noteworthy. The decomposition of the oxy complex proceeds with first order kinetics, indicating that no such complexities as indicated in eqn. (15c) are involved. The decomposition rate is first order with respect to both the colloidal platinum concentration and the partial pressure of H_2, not half order! Based on this kinetic order we are forced to draw the conclusion that

$$(\mathrm{Pt}_x) + \mathrm{H_2} \rightleftarrows (\mathrm{Pt})_x^{2-} + 2\mathrm{H}^+ \tag{18}$$

where $(Pt)_x^{2-}$ is an actual electron donor.

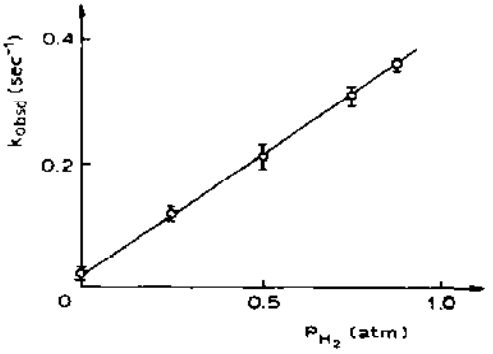


Fig. 4. Dependence of the $P \cdot Fe(II) \cdot O_2 \cdot B$ decay rate on H_2 partial pressure.

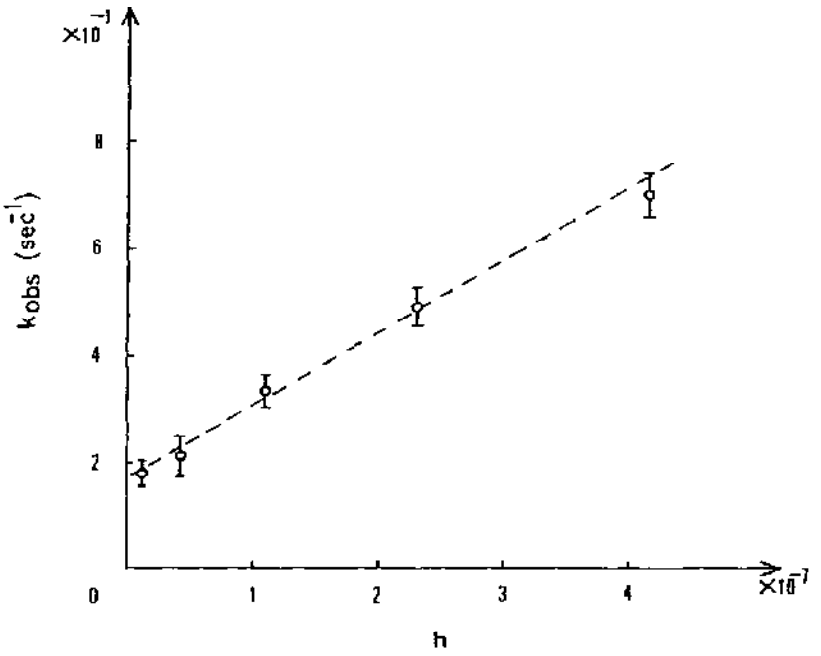


Fig. 5. Dependence of the P·Fe(II)·O_2B decay rate on acidity.

In the next section, a concept of "accelerated and limited two electron transfer" is discussed in detail. From the same viewpoint, colloidal Pt acting as shown in eqn. (18) may be one of the crucial keys. An electron loaded on $(Pt)_x$ is thus formed in a rapid (the rate is faster than the 10^8 M^{-1} s^{-1} rate for the following electron transfer to a porphyrinato–metal(III)!) pre-equilibrium. The following electron transfer, in this case from $(Pt)_x^{2-}$ to the oxy complex, is the rate-determining step in the conversion of the oxy complex to the hydroperoxy complex. There must be an acid-independent as well as an acid-dependent process in the rate-determining electron transfer as clearly shown by the last term in eqn. (17). In other words, electron transfer to the oxy complex is and/or is not assisted by protonation, depending on the acidity of the medium. Thus, the "hidden" part of the mechanism of the reductive O_2 activation was elucidated, as summarized in Fig. 6.

We are now ready to answer the question, "Why are electrons necessary for O_2 activation?". The bond dissociation energies of O=O and related species predict that the electron (as well as a proton) formally weakens the O–O bond as shown in Table 11. Our kinetic studies on O–O bond cleavage, using $T_{piv}PP \cdot Fe(II) \cdot O_2 \cdot B$ reveal that electron transfer really accelerates the O–O bond cleavage by a factor of 4×10^6 (see Table 12). A conclusion drawn therefrom is that the "reductive O_2 activation" must be a very wise and general trick to cleave a very stable O=O bond.

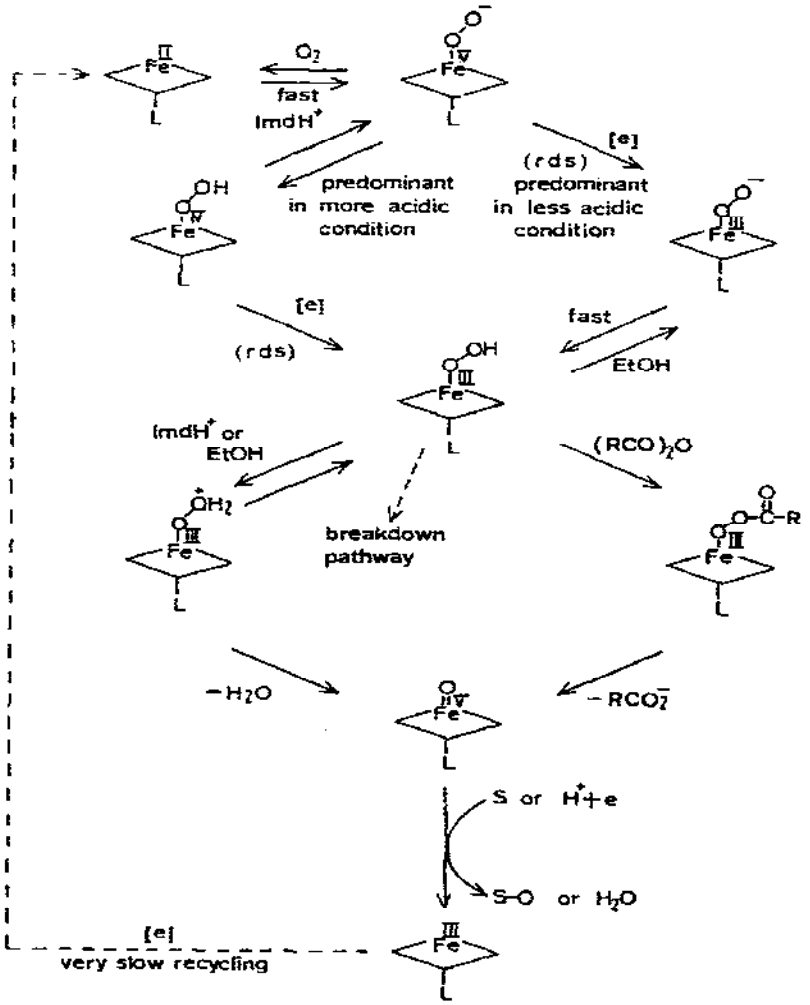


Fig. 6. Reductive O_2 activation mechanism for artificial P-450 system consisting of $T_{piv}PP \cdot Fe(II) \cdot Imd$, $(Pt)_x$, O_2 and H_2.

TABLE 11

Effect of electron and proton transfer on O–O bond dissociation energies

Bond	Energy (kcal mol^{-1})
O=O	118
$O=O^{\cdot-}$	89
$^{\cdot}O-OH$	65
$^{-}O-O^{-}$ (M–O–O^{-})	⩽ 51
R^1O-OCR (M–O–O–CR) ‖ O	⩽ 36

TABLE 12

Acceleration of O=O cleavage by proton and electron transfer

Complex	O–O cleavage rate
$T_{piv}PP \cdot Fe^{II} \cdot (Im, O_2)$	$1.9 \times 10^{-4}\ s^{-1}$
$T_{piv}PP \cdot Fe^{II} \cdot (Im, O_2) + H^+ Im$	$4.4 \times 10^{2}\ M^{-1}\ s^{-1}$
$T_{piv}PP \cdot Fe^{II} \cdot (Im, O_2) + H^+ Im + e$	$1.8 \times 10^{9}\ M^{-1}\ s^{-1}$
$H_2 + col(Pt)_x \rightarrow 2H^+ + 2e + col(Pt)_x$	($[H^+ Imd] = 1 \times 10^{-4}$ M; $[H_2] = 0 \cdot 8$ atom)

Further kinetic information which was highly valuable for the "design of the total molecular system" was obtained. This was information about the product-determining step. Basically, there are two independent and important reactions involved in consuming the metal(V) oxene once formed, reactions (10a) and (10b), which are competitive with each other. This conclusion was drawn since reaction (10b) was shown to be a side reaction.

Based upon the observed turnover number, then for a turnover number of 500, a single reaction of (10b) took place for every 500 reactions of (10a). From the amounts of O_2 and H_2 consumed (the ratio was close to unity) in the closed system, it is easy to estimate how many mols of the oxene were produced per unit time and unit volume (MO). Similarly the mols of epoxide produced per unit time and unit volume (EP) were easily determined. From these two measured quantities, the mols of H_2O produced per unit time and unit volume (W) by the reaction (10c) were estimated as $W = MO - EP$. If other minor side reactions are taken into account, $r = EP/MO$ is the effective consumption ratio of O_2 (or H_2) for the epoxide production.

As expected, the observed r value was quite low and 88.4% of the metal oxene was consumed for useless water production even under high concentration (2.8×10^{-1} M) of olefin and very low concentration (1.3×10^{-7} M) of $(Pt)_x$ [52a], because of the unfavorable competition for a very weak nucleophile, olefin, against a very strong nucleophile, $(Pt)_x^{2-}$. How to overcome this difficult situation and how to design a molecular system behaving just like natural P-450 will be the main topic in the next section.

The present artificial P-450 system, $O_2/H_2/(Pt)_x/P \cdot Mn(III) \cdot B$ or $P \cdot Fe(III) \cdot B$, exhibited interesting possibilities for application to organic synthesis, in spite of the fact that the system still has a very poor r value (effective O_2 or H_2 consumption). Firstly, the present system catalyzed all of the important reactions carried out by the natural P-450; epoxidation, aromatic hydroxylation, aliphatic hydroxylation and t-amine dealkylation (Fig. 7). Epoxidation usually proceeds stereospecifically [50], and for a series of diolefins, chemoselectivity (monoepoxidation predominating under appropriate conditions), regioselectivity (see Table 13) and stereospecificity

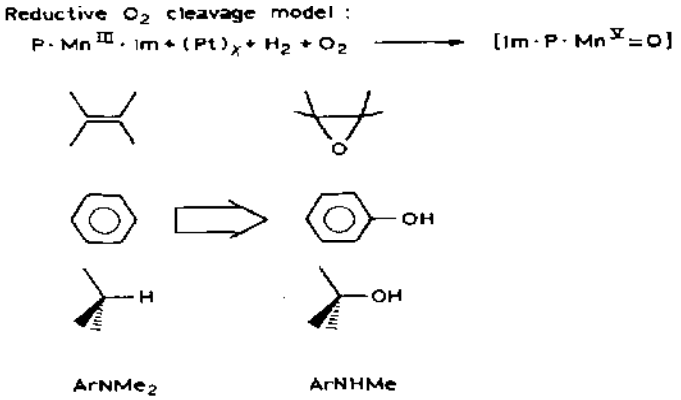


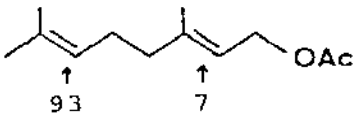
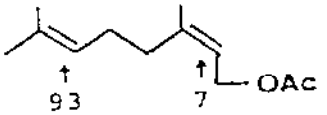
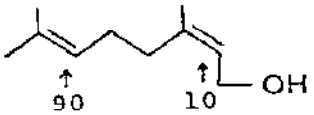
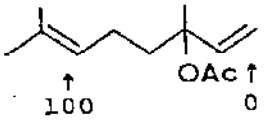
Fig. 7. Important reactions catalyzed by P-450.

were observed [51]. The relative reactivities of a series of olefins were studied, showing good agreement with the PhIO reaction (see Table 14) [50]. Therefore, involvement of the oxene intermediate in the epoxidation is strongly inferred.

Aliphatic hydroxylation did proceed, but only slowly, favoring tertiary hydroxylation. The observed tertiary/secondary C–H relative reactivity was 16.5 (corrected for the number of H, much larger than the usual free radical

TABLE 13

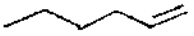
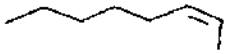
Regioselectivity for mono-epoxidation [a] of diolefins

Diolefin		Turnover (mol prod/ mol cat)
	(Y ~ 100%)	56
	(Y ~ 100%)	41
	(Y ~ 100%)	88
	(Y ~ 100%)	30

[a] For all the examples studied, the epoxidation was stereospecific.

TABLE 14

Relative reactivities [a] of olefins for three different P-450 type reactions

Olefin	Flavin/MeNAH	H_2–Pt/PVP	PhIO
	1	1	1
	< 0.05	< 0.05	< 0.05
	0.97	0.88	0.96
	0.10	0.09	0.09
	1.5	1.4	1.3

[a] $\frac{V_i}{V_j} = \frac{k_i K_i (1 + K_j [S]_j)[S]_i}{k_j K_j (1 + K_i [S]_i)[S]_j}$.

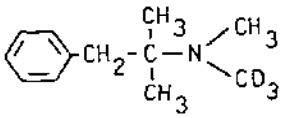
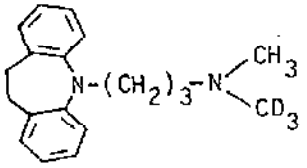
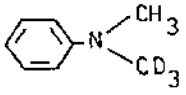
substitution where the ratio is 3–5 [59]). The large tert/sec ratio strongly suggests that the P-450 type intermediate is considerably electrophilic (electron deficient) [50].

Benzene, naphthalene and substituted benzenes were hydroxylated but much less efficiently than olefins [57,60]. An interesting observation here was the effect of an inorganic acid or organic acyl anhydride on the product distribution from toluene. When the concentration of the acid or acyl anhydride was high, the ring hydroxylation predominated. A reasonable explanation for the effect may be that acid or acyl anhydride converts the hydroperoxy complex to the P-450 intermediate (16), and the process competes with spontaneous O–OH homolytic scission inducing autoxidation [52b]. Another observation was a rather small (but positive ($\rho > 0$)) effect of a ring substituent on the relative reactivities of substituted benzenes, in accord with the electron-deficient radical mechanism.

A relatively strong inhibitor for the $(Pt)_x$ activity was found to be an aromatic phenol, which spontaneously quenched the reaction at the monohydroxylation stage. Dealkylation of tert-amine proceeded very efficiently [47], as in other model systems [28]. The observed turnover number went to at least 200 and reached more than 1000. From detailed analysis of the intermediates formed, the initial formation of α-hydroxylated amine was strongly suggested [47] as concluded in the literature [61]. However, the initial reaction seems to be a one electron transfer from a tert-amine to the artificial P-450 intermediate as shown by the magnitude of the kinetic k_H/k_D (see Table 15). Coincidence between this value of k_H/k_D with that

TABLE 15

Kinetic isotope effect for P-450 and related oxidations

Substrate	Oxidation method	k_H/k_D	Ref.
	cyt. P-450	1.8 ±0.2	48a
	Rat liver microsome Anodic oxidation	1.64±0.05 1.88±0.06	48b
	$TPPMn^{3+}$–Pt–H_2–O_2	1.71±0.02	47

for natural P-450, further supports the involvement of the oxene intermediate.

For the practical application of the artificial P-450 as a catalyst for epoxidation or hydroxylation, immobilization of the porphyrinato metal or related complex onto a synthetic polymer has been attempted. Interestingly, the catalysis was effective but serious decomposition of the catalyst molecule was reported. Decomposition was serious even for halogenated porphyrins or phthalocyanines [58] which had been reported to last a very long time. It seems probable that the minor decomposition of these "stable catalysts", not detected by former workers, takes places in homogeneous solution.

(iii) MO_2 species

In the reductive activation of oxygen, either with natural or artificial P-450, a very strong O=O bond is cleaved quickly. However, the mechanism is incredibly simple: (1) rapid electron supply to the "resting" P · Fe(III), (2) strong O_2 binding by Fe(II) (much better than Mn or Co [31,62]), and (3) further electron supply and proton supply! The P · FeO_2H formed in the first stage, (1)–(3), is, interestingly, exactly the same as that coming from O atom transfer when H_2O_2 is used [18,52]. Therefore, the unique and important process must involve (1)–(3) as we are constantly testing the efficiency of the electron channel in the "P-450 total system" [42,50–52,60,63].

E. THE ACCELERATED AND LIMITED TWO ELECTRON TRANSFER IN REDUCTIVE O_2 ACTIVATION; THE APPROACH TO AN ARTIFICIAL SUPERENZYME

Based on detailed kinetic studies of the simple artificial P-450 type O_2 activation molecular system, we solved the important problem of the useless reductive decomposition pathway of the metal oxene once formed, as discussed in the previous section. The situation we are now facing is rather complicated; there are two self-contradictory requirements for possible solution—(i) an electron must be transferred to P · M(III) quite rapidly to gain a good turnover frequency (mol product/mol catalyst unit time) while (ii) a (further) electron must be transferred to P · M(V)O extremely slowly to prevent the useless oxene consumption (see Fig. 8).

However, the enzyme P-450 solves the problem; maybe the total P-450 system does that. Let us reconsider the mechanism of electron transfer control led by the enzyme system, by focusing our attention on their structure and "molecular organization". As schematically drawn in Fig. 9, their "molecular organization" is very significant. Maybe the flavin moiety (Fl) in the reductase accelerates one electron transfer from the NAD(P)H bound to the reductase to the porphyrinato Fe(III) in the P-450 active site via P-450–reductase (temporary) association. Thus, the overall rate determining production of P · Fe(II) may be very much accelerated. Whenever a substrate is bound to the P-450 binding site, the resting P · Fe(III) low spin state may be converted to the high spin state, allowing electron transfer. O_2 binding to P · Fe(II) is reasonably fast and a subsequent one electron transfer to P · Fe(II) · O_2 must be fast, since the total molecular system still keeps one nearly free electron $(NAD(P) \cdot Fl)^{\cdot}$. We need two protons for the

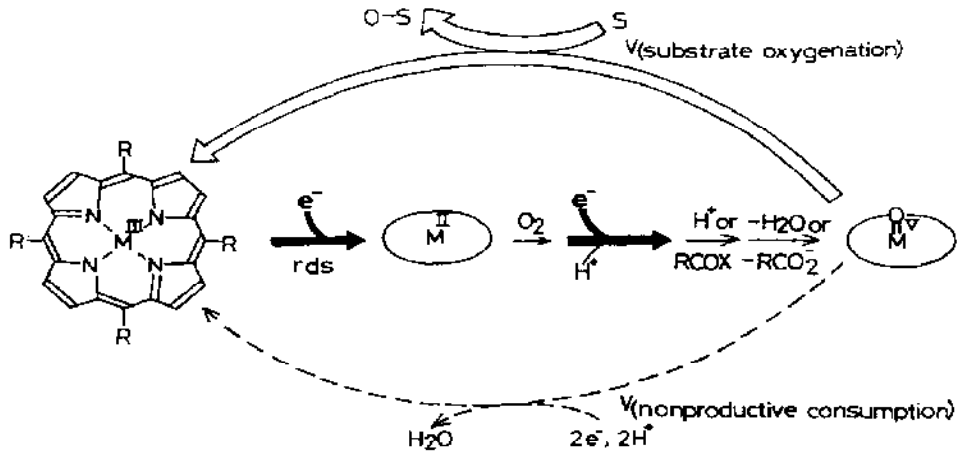


Fig. 8. Concept of accelerated and limited two electron transfer.

Nature's Trick

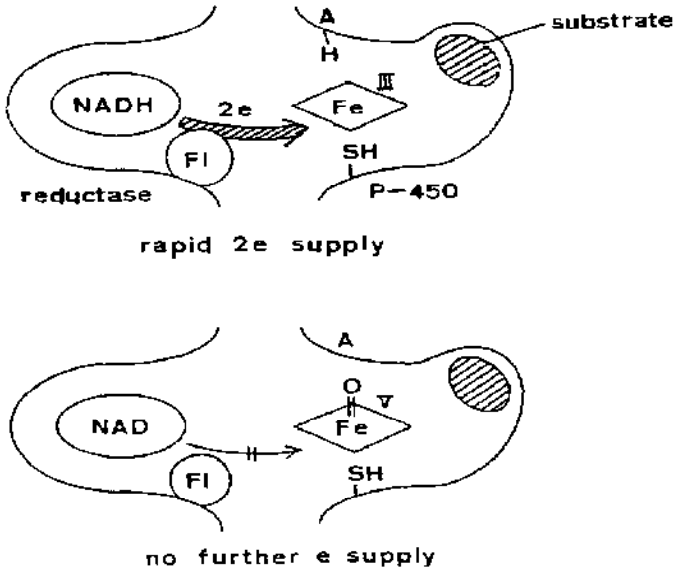


Fig. 9. Accelerated and limited two electron transfer in P-450/reductase couple.

O–O cleavage, but they may easily be supplied from bound water or even from the enzyme wall; we have learned from our model that in aqueous solution of pH less than 7, protons were spontaneously and very rapidly supplied; then P · M(V)O may be formed. The beauty of the system is that now there are no more available electrons, since NAD(P)H is converted to NAD(P) after two electron transfer. We may reasonably assume that dissociation of NAD(P) from the reductase is much slower than the electron transfer (10^8 s^{-1} M^{-1} even for the simple artificial system) and related reactions. Therefore, there may be enough time for the bound substrate to pick up an active O atom from the P-450 reaction center nearby.

If the situation is as simple as just described, i.e. (a) substrate binding—spin control and (b) accelerated and limited two electron transfer,

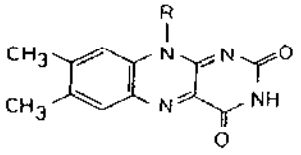
TABLE 16

Flavin catalyzed electron transfer from dihydronicotinamide to porphyrinatometal(III)

System	Rate constant (M^{-1} s^{-1})
MeNAH + O_2	0.00035
Mn^{III}·TPPS + MeNAN	0.1
Mn^{III}·TPPS + MeNAH (catalyzed by 1.0×10^{-4} M FMN)	160

the design and synthesis of a total molecular system mimicking the P-450 molecular system is an achievable challenging target for chemists.

Firstly, we have proven that flavin really accelerated one electron transfer from dihydronicotinamide to the artificial porphyrinato Mn(III) or Fe(III) in a homogeneous solution (Table 16). This is not only a remarkable acceleration effect on the rate-determining one electron transfer but also a remarkable, unexpected increase in the r value (EP/MO) [63]. In the presence of FMN (flavin mononucleotide, **6**) the total system (*meso* tetraphenyl-*p*-sulfonate porphyrinato Mn(III) (TPPS · Mn(III)), N · Me di-



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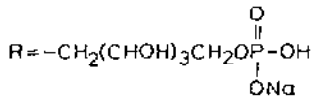
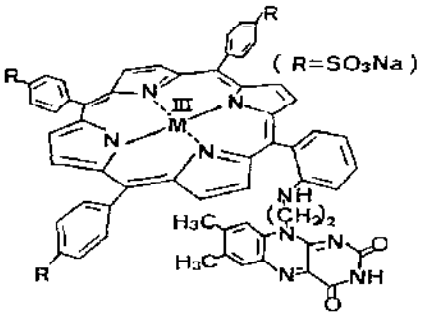
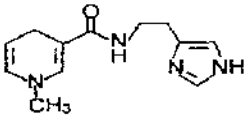


hydronicotinamide (MeNAH), and *N*-methylimidazole (*N*-MeIm), O_2 and H_2O) provided an r value of 0.5 which is much larger than the previous total system (TPPS · Mn(III), H_2, $(Pt)_x$, O_2 and H_2O) which has an r value of 0.05. This remarkable improvement, although fortuitous, may be interpreted as follows; since the O_2 cleavage reaction consists of a series of very fast elementary steps, $FMN^{\cdot}$ or FMN produced after one or two electron transfer may remain, with some probability, near the reaction center, protected by a solvent cage. It may take some time to replace these with a fresh MeNAH or $FMNH^{-}$. This situation provides the necessary requirements for the "accelerated and limiting two electron transfer" [64]. Our primary success in using FMN, encourages us to design a more sophisticated molecular system to fit the requirements for the "accelerated and limited two electron transfer".





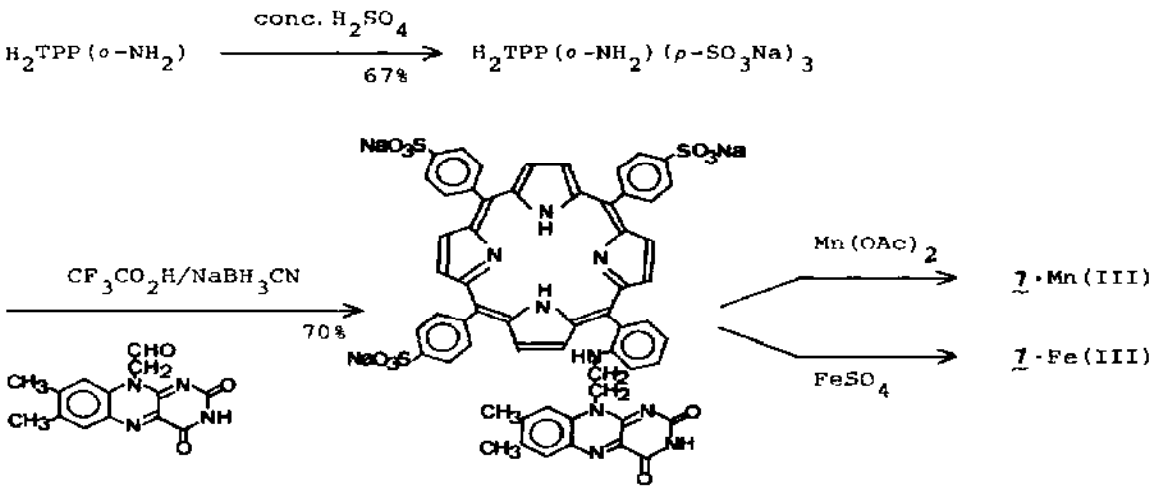


Fig. 10. Synthesis of flavoporphyrin.

The molecular system, shown in 7, was synthesized in a straightforward fashion as shown in Fig. 10. As expected, a remarkable rate enhancement for the rate-determining P · Mn(III) or P · Fe(III) reduction was observed. A satisfactory r value of 0.2 was also observed. These successes derive from "self-organization" between the reaction center, P · M(III), and the electron source RNAH-Im via imidazole coordination to the central Fe(III) or Mn(III). These excellent indices strongly suggest that the P-450 type catalytic activities must be there. This is the case, as shown in Table 17, for a typical example. Even in their "premature" stage, a well-designed self-organizing molecular system showed superenzymic activity: better turnover

TABLE 17

Comparison of epoxidation efficiency between native and artificial P-450 type molecular systems: superenzyme activities

Parameter	
Electron flux (rds)	$M^{III} \rightarrow M^{II}$
artificial	$0.45\ s^{-1}$
native	$0.2\ s^{-1}$
Turnover freq.	$CH_2{=}CH_2 \longrightarrow$ 
artificial	$11\ m^{-1}$ mol product (mol catalyst)$^{-1}$ min^{-1}
native	$4\ m^{-1}$ mol product (mol catalyst)$^{-1}$ min^{-1}
Turnover	
artificial	700 mol product (mol catalyst)$^{-1}$
native	250 mol product (mol catalyst)$^{-1}$

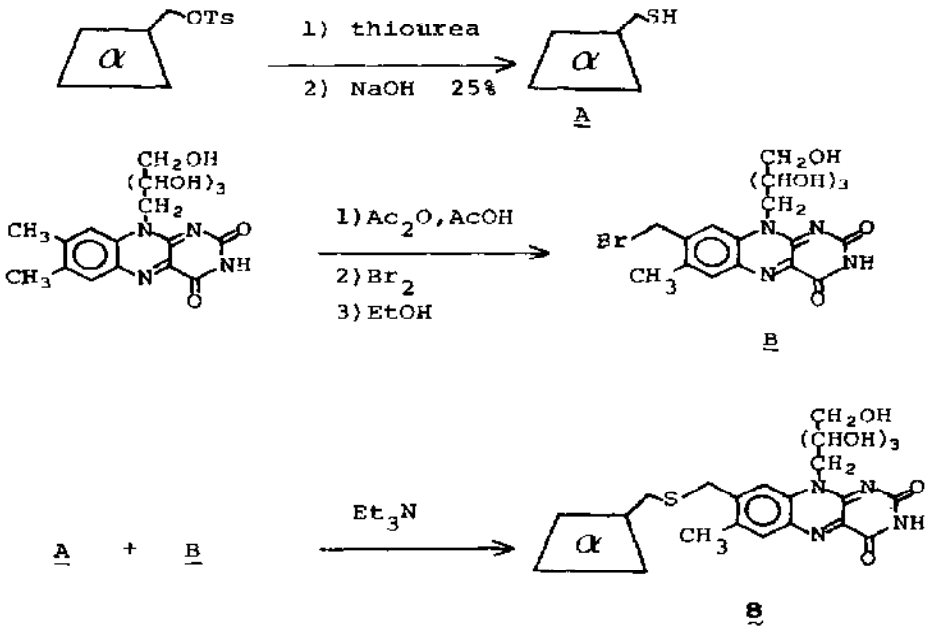
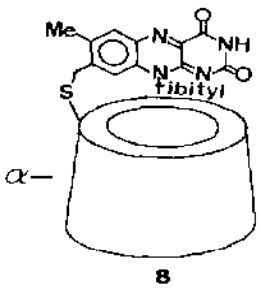


Fig. 11. Synthesis of flavo-α-cyclodextrin.

number, better turnover frequency, faster rate-limiting electron transfer constant.

Then, an important question may arise, "Is this the limit for the artificial enzyme system?". The answer is, "No, not yet", because we know that: (i) the imidazole–P · Mn(III) association constant is not large enough in water (40 M^{-1} for MeIm) to give saturation at the low imidazole concentration; and (ii) the off rate of RNA^+–Im (very similar to RIm) is still small, 10 s^{-1}, which may limit the turnover frequency.

A plausible solution to the question may be the use of a more rapidly equilibrating, more strongly binding "host"–guest combination. Since RNAH is an ideal guest providing two electrons to the reaction center, RNAH must not be changed and an appropriate host must be designed. Flavocyclodextrin (FlCD) (**8**) was thus prepared according to Fig. 11. The FlCD showed a satisfactorily strong binding of RNAH with an associa-



8

TABLE 18

Electron transfer activities of native and artificial flavoenzymes

$$RNAH + Fl \overset{k}{\rightleftarrows} RNA^{\cdot} + Fl^{\cdot -} + H^{+} \overset{H^{+}}{\rightleftarrows} RNA^{+} + FlH_2$$

where $k = 50\ M^{-1}\ s^{-1}$

	k_{cat} (s^{-1})	k_{cat}/K_M ($M^{-1}\ s^{-1}$)
Flavoenzyme	15.5	326000
Flavopapain	5.4×10^{-3}	56
FlavoCD	0.5	1200

tion constant of 2500 M^{-1} for n-hexylNAH (HxNAH) or using 4×10^{-4} M HxNAH, ca. 50% of the cyclodextrin molecules were occupied by HxNAH. More interestingly, the electron transfer from RNAH to Fl proceeded quite effectively in the molecular system. Although the present rate constant for the electron transfer

$$RNAH + Fl \rightleftarrows RNA \cdot Fl^{-} \cdot H^{+} \rightarrow RNA^{+} + FlH_2 \qquad (19)$$

is smaller by a factor of 31 than that for native flavoprotein, it is greater by a factor of 21 than that for the structurally related flavopapain [65] (see Table 18). Based on these rate constants, the "self-organization" in the present RNAH/FlCD molecular system seems to be close to the best and if this is so, then interestingly, the strict "preorganization" frequently discussed in the field does not seem essential. Another interesting aspect in the present RNAH/FlCD system is, as expected, fast on- and off-rates of the guest. This does not require "on- or off-rate control", rather the very much accelerated electron transfer must still be rate determining. This accelerated NAH–Fl electron transfer capacity of the present molecular system naturally reflects in the accelerated electron transfer to the reaction center P · M(III) (see Table 19).

A better "molecular system" for faster electron transfer from RNAH to P · M(III) was then made. The target molecule (**9**) was prepared as shown in Fig. 12. This showed a further acceleration by a factor of 5.5 in electron

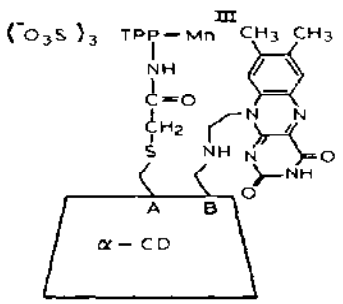
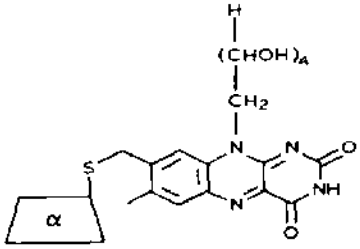


TABLE 19

Reduction rate of Mn^{III}porphyrin at 25 °C, pH 7.4

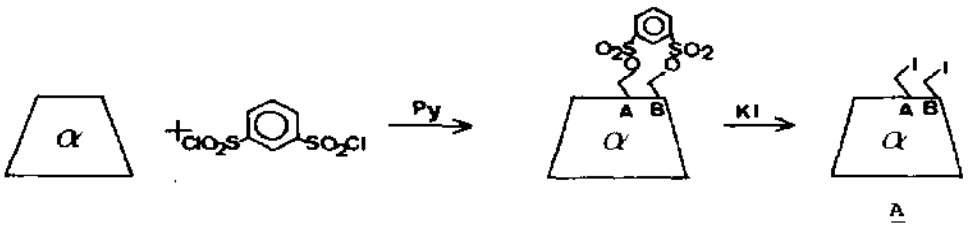
Mn^{III}porphyrin (8×10^{-6} M)	Flavin (8×10^{-6} M)	RNAH	k_2 [a] ($M^{-1}\,s^{-1}$)
Mn^{III}TPPS	FMN	MeNAH	36
Mn^{III}TPP($SO_3Na)_3$(FL)		Im-NAH	110 [b]
Mn^{III}TPPS		n-hexyl-NAH	200

[a] $v = k_2[Mn^{III}TPPS][RNAH]$ in H_2O.
[b] In 50% aq. EtOH.

transfer from HxNAH to P · Mn(III), again without strict preorganization. These data strongly suggest that easily synthesized "self-organization" molecular systems play satisfactory roles and may be used more conveniently than the time-consuming strict preorganization molecular system.

The remaining question to be answered is "spin control" (from low to high spin) by substrate binding. Natural P-450 does that, probably via extrusion of some water molecules. Some water molecules seem to be present in the enzyme cavity near the reaction center where the enzyme does not bind the guest, and where the central metal(III) has H_2O and S^- (from the enzyme wall) to interact from both apical sides. Under these circumstances, the low spin state may be the more favored. When a hydrophobic guest is bound to the cavity, these water molecules have no place to interact with the central metal (Fig. 13), as clearly demonstrated by X-ray crystallography (Fig. 14). However, we need more information to convince ourselves about the spin-control mechanism.

A plausible molecular system design is shown in Fig. 15, where the transannular positions of the porphyrin ring were attached to the A and D sites (transannular sites) of CD to give little internal rotation freedom of the porphyrin moiety [66]. The molecular cavity of β-cyclodextrin seems to accommodate some water molecules, as ascertained for the parent β-CD by X-ray crystallography (Fig. 16) [67]. "Spin control of porphyrinato Fe(II)" by water soluble adamantane carboxylate was observed, judging from the corresponding shift in λ_{max} in an aqueous solution. Therefore, water extrusion seems to be a rather general mechanism for "spin control", which is



$H_2TPP(o\text{-}NH_2)(p\text{-}SO_3Na)_3 + (SCH_2COCl)_2 \xrightarrow[64\%]{}$

$H_2TPP(o\text{-}NHCOCH_2SSCH_2CO_2H)(p\text{-}SO_3Na)_3 \xrightarrow[80\%]{DTT} H_2TPP(o\text{-}NHCOCH_2SH)(p\text{-}SO_3Na)_3$

B

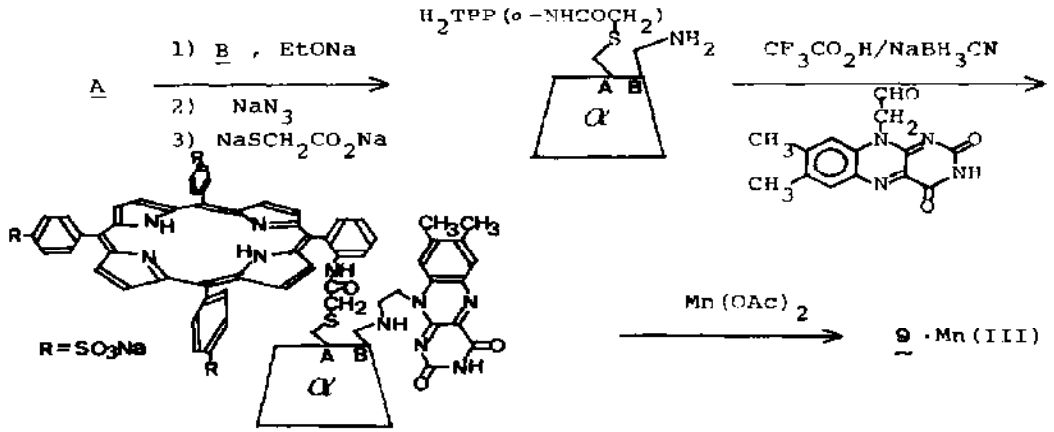


Fig. 12. Synthesis of A,B-flavoporphyrino cyclodextrin.

then to be reflected in large (10^2 fold for natural P-450) rate enhancement in the rate-determining electron transfer.

All of these new observations directly lead to the design of a very convincing molecular system, where the target molecule is shown in Fig. 17, as a typical example. The artificial molecular systems, some of which have already shown "superenzymic" activity in their premature stages, will show much better catalytic properties than the natural molecular system, P-450/P-450 reductase/NAD(P)H.

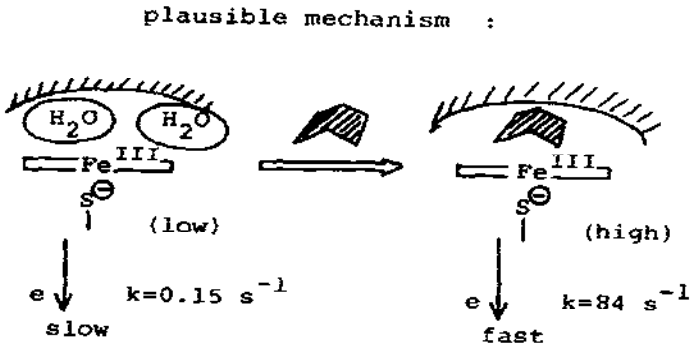


Fig. 13. A plausible mechanism of spin control by substrate binding.

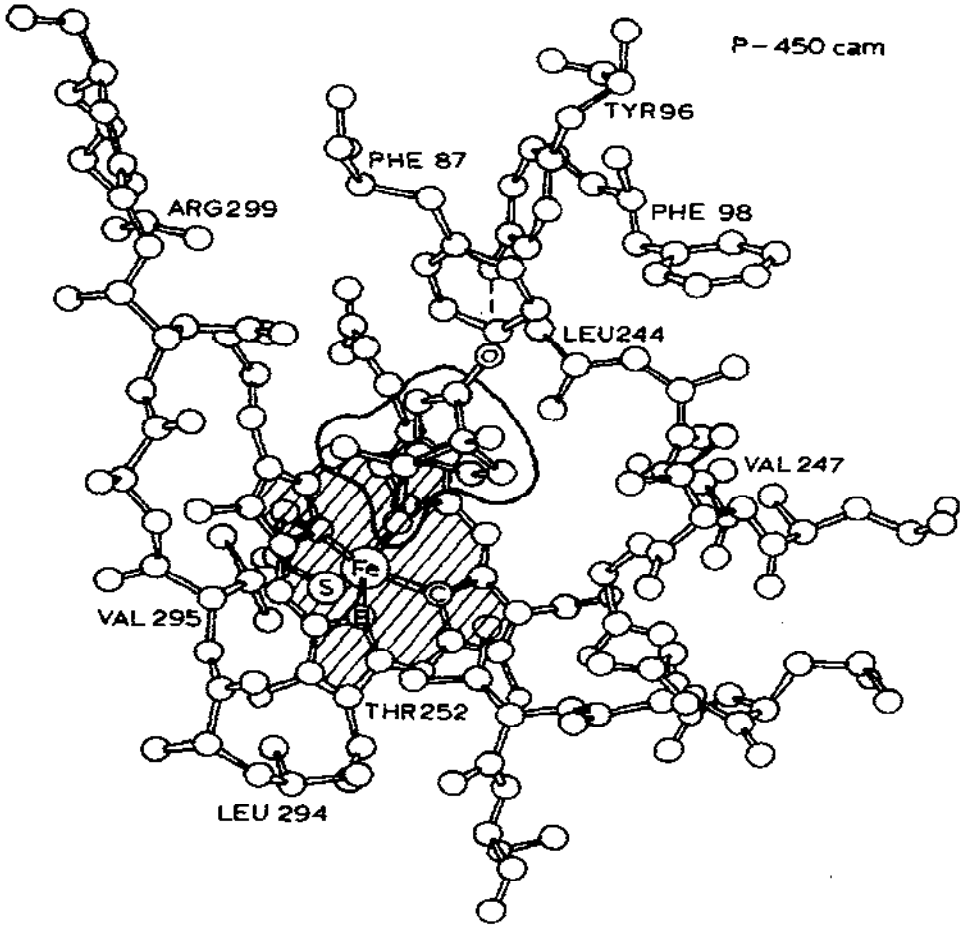


Fig. 14. X-Ray crystallography of the cytochrome P-450_{cam} active site.

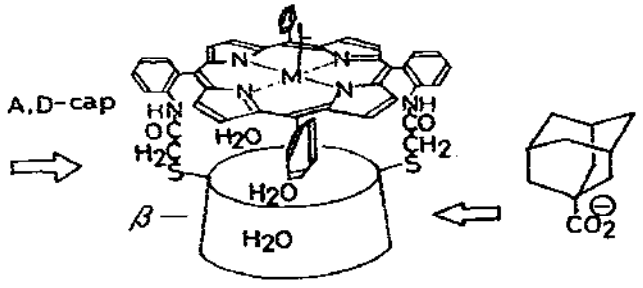


Fig. 15. Transannularly capped β-CD with porphyrin as spin control model.

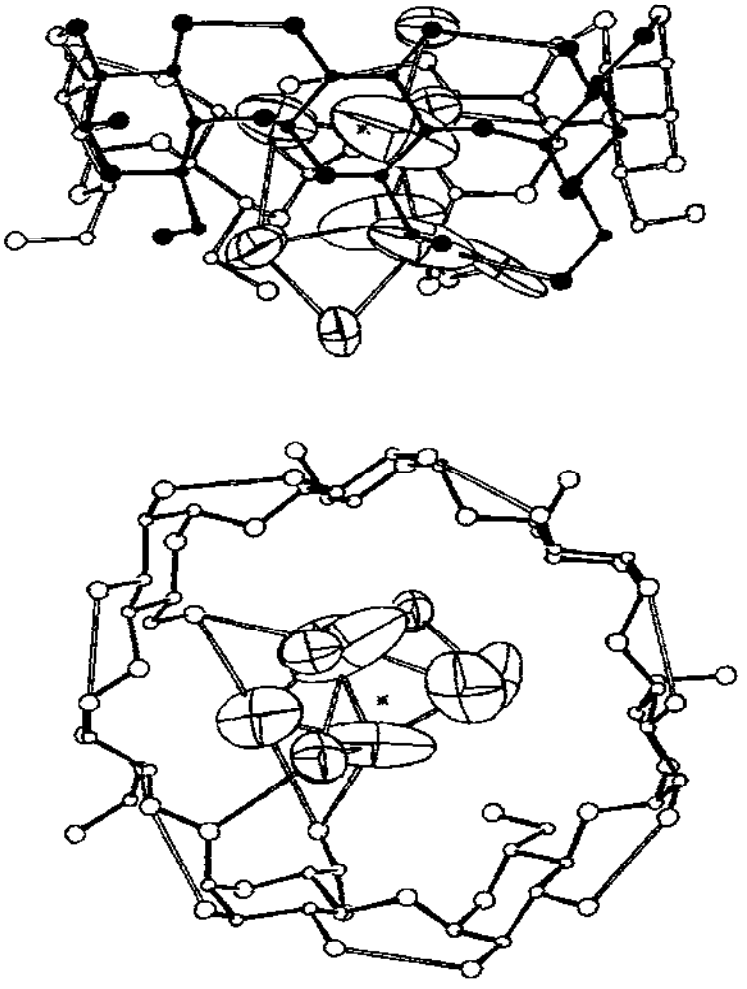


Fig. 16. X-Ray crystallography of β-cyclodextrin hydrate.

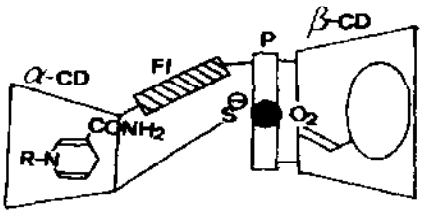


Fig. 17. Total molecular model of artificial P-450 superenzyme. Black circle is central metal, Fl is flavin, large and small buckets are β- and α-cyclodextrins, respectively.

We have been deeply involved in asking why a complex enzyme system is needed to activate an otherwise very stable O_2 bond efficiently. It may also be worthwhile to consider briefly why nature uses the porphyrin–iron complex in its molecular system and how their "molecular devices" operate correctly.

Interestingly, Mn(III) quite efficiently catalyzes monooxygenation through the reductive O_2 activation mechanism in the artificial molecular system. Often Mn(III) catalysis is even better than Fe(III) catalysis under normal conditions.

However, if we take the somewhat special local conditions under which the enzyme system works, we are forced to recognize how much better is Fe(III). There may be three important factors to consider: O_2 affinity, intermolecular (most probably outer sphere) one electron transfer, and binding with an S^- ligand. The O_2 affinity of P · Fe(II) is widely known to be much higher than the corresponding P · Mn(II) complex, as typically exemplified by Table 20. However, interestingly, at atmospheric pressure, where any artificial enzyme is usually used, the low affinity Mn(II) does bind O_2 almost as well as the high affinity Fe(II) complex (30% and 99%, for

TABLE 20

O_2 affinities of Mn(II) and Fe(II) porphyrins [a]

Complex		Partial pressure (mm Hg)	Temperature (°C)
O_2 affinity			
TPP · Fe^{II} · Im	$P_{1/2}$	5	−45
TPP · Mn^{II} · Im	$P_{1/2}$	900	−78
Liver	P_{O_2}	40	36

Oxy complex (%)	P_{O_2}	Mn	Fe
	760/2	30×	99
	40	4	89

[a] At high P_{O_2}, the catalytic activity of Mn is better than that of Fe.

TABLE 21

O_2 formation and rebinding

O_2^- leakage	
$TPP \cdot M^{II} \cdot Im + O_2 \xrightarrow{k} TPP \cdot M^{III} \cdot Im + O_2^-$	
k (M^{-1} s^{-1}) Fe^{II}	Very small
Mn^{II}	1×10^5
O_2^- rebinding	
K (M^{-1}) Mn^{II}	2.8×10^5

TPP · Mn(II) and TPP · Fe(II), respectively), not making any significant difference in the overall monooxygenation catalysis in the artificial system. However, in biological circumstances where O_2 partial pressure may be as low as 40 mm Hg, the remarkable difference between the O_2 affinities of the two metal(II) ions is directly reflected in the actual O_2 binding efficiency. Therefore, P · Fe(II) is more effective by a factor of 47 than P · Mn(II) and the former might survive in the natural selection.

The second factor is related to the first factor; outer-sphere one electron transfer from P · Mn(II) to O_2 is one of the faster reactions involved (see Table 21). Of course, there is a "rebound" mechanism in which most of the O_2^- once formed is tightly bound to another P · Mn(II). Still some O_2^- (well known as a strongly toxic compound toward living systems) leakage must take place.

The third factor, the combination of P · Fe(II) and S^- ligand also seems to be important as discussed below; but if P · Fe(II) is to be selected (e.g. for the reasons mentioned above) in natural selection, then from the binding problem, S^- has also to be selected as the sole possible partner.

The binding factor may best be described by an MO calculation on a simplified model system. Yamaguchi et al. carried out an MO calculation of $L \cdot (NH_3)_4M(V)$ oxene and obtained a rather simple correlation between "electron donation capacity" of L and the net charge appearing on the terminal O atom. As expected, Mn and Fe do not fall on the same line (see Fig. 18) [68]. We now know that terminal O is electron-deficient, based on the observed effect of substituents in the benzene ring on the relative rate of hydroxylation. Similar information is also provided from epoxidation of olefins and even from hydroxylation of alkanes. If this observed electron deficiency at the terminal O is taken into account, a significant conclusion may be drawn; i.e. the terminal O in the Mn(V)–N combination and in the Fe(V)–S^- combination behave similarly. The Mn(V)–S^- combination does not provide a sufficiently electron deficient terminal O which is probably much less reactive, while the Fe(V)–N combination may provide a two

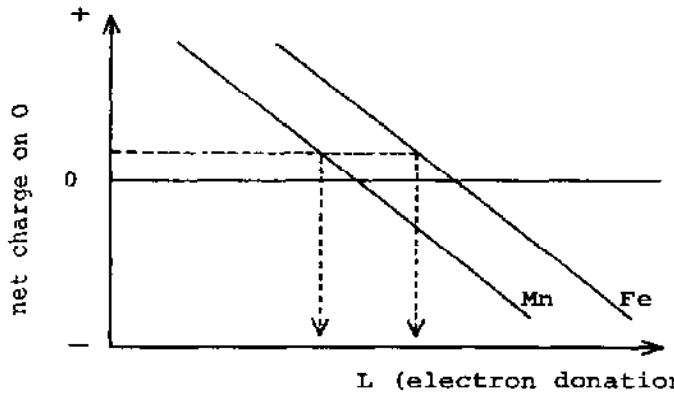


Fig. 18. Ligand–central metal combination for appropriate electronic state of oxene. S-Fe and H-Mn may be suitable couples.

electron deficient terminal O, which may attack itself (intermolecularly or intramolecularly). In the artificial molecular system, neither the Fe–N nor Mn–S^- combination is a good catalyst for P-450 monooxygenation.

There are some interesting replacement experiments for natural P-450 systems, in which Fe and Mn, the most frequently used central metal ions, are replaced by other metal ions (Cr, Ru, Mo, V and others) [69]. Those examples will provide clearer answers for the problem of metal–ligand combination in P-450 chemistry.

F. FUTURE ASPECTS

Before closing the review, it is necessary for the author to suggest a few future directions for P-450 enzyme chemistry. One, in the short range, is a detailed understanding of the enzyme mechanism, based on the knowledge provided by recent X-ray crystallography [70]. Structures of short-lived intermediates (such as oxene-substrate adduct or P · M–O·) will be progressively elucidated by the use of new techniques.

Another interesting aspect, in the longer range, is P-450 as a molecular device. This is located between chemistry and physics. From the viewpoint of electron flow and the control system found in the artificial and natural P-450s, the P-450 system (enzyme, electron, O_2, and a signal compound) looks like a good candidate for a molecular device.

Relating to the original biological functions of the P-450 enzyme in liver, the detection and cure of certain diseases are also important future aspects of P-450 chemistry.

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