A Chemical Model of Cytochrome P-450: Mono-oxygenase-like Activation of Dioxygen

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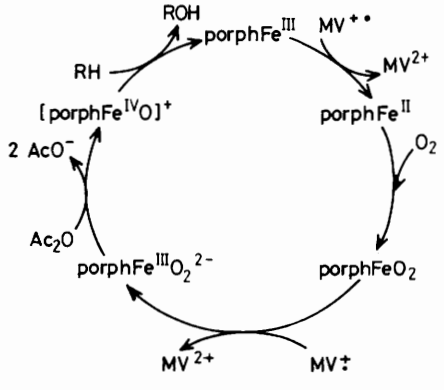
A new model for cytochrome P-450 activation of O_2 on iron porphyrin as a catalytic centre in the presence of Zn(Hg) as a reducing agent, methylviologen as a mediator, and acetic anhydride as an acylating agent is proposed.

Cytochrome P-450 is a typical mono-oxygenase, inserting one oxygen atom of O_2 into a substrate molecule and using the other to form H_2O.[1] Sligar *et al.*[2] obtained evidence for the initial acylation and cleavage of the O–O bond, prior to water formation, in the system P-450 + $^{18}O_2$ + dihydrolipoic acid. Simple systems modelling the active centre of P-450 have been prepared from the π-cation-radical $[(porph)FeO]^{\cdot+}$,† formed by oxygen atom transfer from peracids[3] and by acylation of haem peroxo complexes[4] by acetic anhydride.

In order to construct a better model of O_2 activation by P-450 two stages must be united in one system: the reduction of O_2 co-ordinated on haem to a peroxo complex and the acylation of the latter with cleavage of the O–O bond. The synthesis of the peroxo complex by reduction of co-ordinated dioxygen has been achieved recently in aprotic media at low temperatures[5] and using sterically hindered TpivPPFeBr.[6–8] The first example of electrochemical reductive O_2 activation has also been reported, using haem, supported at the cathode surface, and an acylating agent.[4,9]

This communication deals with reductive O_2 activation at Fe–porph by zinc amalgam in the presence of methylviologen ($MVCl_2$) as an electron transfer agent, and acetic anhydride as an acylating agent, in acetonitrile. In the absence of the porphyrin complex no noticeable O_2 absorption takes place indicating that the cation-radical formed from $MVCl_2$ is stable in an O_2 atmosphere in this solvent. Addition of Fe–porph to the mixture leads to O_2 absorption and oxidation of the substrate (Table 1).

In the absence of $MVCl_2$ the reaction is about 100 times slower. The absence of any other component stops the substrate oxidation completely. In the process of cyclohexane



Scheme 1. Catalytic cycle for the cytochrome P-450 model system [Zn(Hg) + $MVCl_2$ + porphFe + O_2 + Ac_2O + RH].

† Abbreviations: porph = porphyrin, TPP = tetraphenylporphyrin, TF_5PP = tetra(pentafluorophenyl)porphyrin, TpivPP = tetra-(α,α,α,α-*o*-pivalamidophenyl)porphyrin, all porphyrins are dianions; MV^{2+} = methylviologen dication.

Table 1. Oxidation products of the catalytic system Zn(Hg) + $MVCl_2$ + TPPFeCl + Ac_2O + RH.[a]

Substrate RH	Product (conc./mM)		Recycling number mol prod/mol cat	O_2 adsorbed /μmol	Yield per O_2 absorbed/%
$PhNH_2$	p-$HOC_6H_4NH_2$	(2.7)	9	100	3
$PhNMe_2$	HCHO	(3.3)	11	24	18
$PhOCH_3$	HCHO	(1.7)			
	PhOH	(1.8)	14	68	8
	o-$HOC_6H_4OCH_3$	(0.7)			
$PhOCD_3$	HCHO	(0.3)			
	PhOH	(0.3)	5	85	2
	o-$HOC_6H_4OCD_3$	(0.8)			
$\overline{CH_2[CH_2]_4CH_2}$	$\overline{CH_2[CH_2]_4CHOH}$	(3.0)	10	36	12
		(7.0)	25	32	30
		(8.3)	33	51	25
cis-$\overline{CHMe[CH_2]_4CHMe}$	*cis*-$\overline{CHMe[CH_2]_4CMeOH}$	(3.2)			
	trans-$\overline{CHMe[CH_2]_4CMeOH}$	(0.7)	67	18	
	sec. 1,2-Dimethylcyclohexanols	(5.5)			

[a] *Reaction conditions:* $MVCl_2$ (0.1 mM), Ac_2O (10 mM), RH (1 M), TPPFeCl (0.3 mM), MeCN (1 ml, solvent), 20 °C, 30 min. [b] With $TF_5PPFeBr$. [c] With TpivPPFeBr.

Table 2. Comparison of chemical model systems with cytochrome P-450.

System	Retention of configuration[a]/%	Regioselectivity: tert.: sec.: prim.	Kinetic isotope effect: k_H/k_D[b]
Zn(Hg) + $MVCl_2$ + TPPFeCl + O_2 + Ac_2O	60	2.8 : 1[a]	7
$[TPPFeO_2]^-$ + Ac_2O [c]	60—70	3.3 : 1 : 0.8[d]	7
Cytochrome P-450	100[e]	7.5 : 1 : 0.07[d,e]	7—8[f]

[a] Oxidation of *cis*-1,2-dimethylcyclohexane (tert. : sec.). [b] Hydroxylation of anisole. [c] Data from ref. 4. [d] Oxidation of isopentane. [e] Data from ref. 10. [f] Data from ref. 11.

oxidation the Fe–porph complex is partially consumed. For TPPFeCl, $TF_5PPFeBr$, and TpivPPFeBr the loss corresponds to 50, 5, and 15%, respectively. 1 mol of Ac_2O is consumed and 2 mol of acetate formed per mol of O_2 absorbed. In cyclohexane oxidation with $TF_5PPFeBr$, the reaction stoicheiometry is $ROH : O_2 : Ac_2O = 0.3 : 1 : 1$. Compared with P-450 oxidation, less ROH is formed per mol of O_2 and Ac_2O consumed, probably owing to side oxidation reactions of Fe–porph and $MVCl_2$ in the model system.

In the somewhat similar system described recently by Tabushi[7] reductive O_2 activation was achieved with $Pt+H_2$ as a reducing agent and benzoic anhydride as acylating agent. Cyclohexene is oxidized to the epoxide in only 5% yield per mol of O_2 absorbed. The comparison of our catalytic system with a stoicheiometric reacting system and the P-450 system shows important similarities (Table 2). It may be concluded that the same active unit, presumably $[(porph)FeO]^+$ is active in all three systems, hydroxylating the C–H bond in a non-radical fashion.[4]

In order to determine the type of O–O bond cleavage occurring in the catalytic system, dioxygen labelled with ^{18}O (47%) was used, and the products were analysed by mass spectroscopy. The cyclohexanol and acetate produced contained $C_6H_{11}{}^{18}OH$ and $CH_3CO^{18}O^-$ (40 and 27%, respectively). Thus mono-oxygenase-type O–O bond cleavage was essentially confirmed. The catalytic cycle for the model system may be represented in a similar way to that of P-450 (Scheme 1).

The rate of cyclohexane oxidation in the presence of TpivPPFeBr is 1.1 cycle/min, the rate controlling step being the generation of $MV^{+\bullet}$. In the Tabushi system,[7] the rate of cyclohexene oxidation is 0.8 cycle/min for TpivPPFeCl. The rate of cyclohexane oxidation in the native P-450 system is 8.8 cycle/min.[12]

Thus, the catalytic system described here for the hydroxylation of C–H bonds in alkanes behaves similarly to native cytochrome P-450.

Received, 24th October 1986; Com. 1525

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