CROATICA CHEMICA ACTA CCACAA 49 (2) 223—235 (1977)

CCA-994

YU ISSN 0011-1643
577.15.087.4:541.124
Conference Paper
(Authors' Review)

Mechanism of Oxygen Activation in Hydroxylation Reactions Involving Cytochrome *P450**

A. A. Akhrem, D. I. Metelitza, S. M. Bielski, P. A. Kiselev, M. E. Skurko, and S. A. Usanov

Institute of Bioorganic Chemistry, BSSR Academy of Sciences, Minsk, USSR

Received October 12, 1976

In the 20—37 °C range the kinetics of cyclohexene epoxidation, naphthalene and cyclohexane hydroxylation and oxidative demethylation of a group of amines in the presence of rat liver microsomes, NADPH and O_2 has been studied.

There is a linear relationship between the activation enthalpy of the decomposition for the substrate-cytochrome *P450*-O_2 complex, ΔH^*, and the activation entropy ΔS^*:

$$\Delta H^* = 20.7 + 336\,\Delta S^*$$

Oxidation of the same substrates at 20—37 °C has been carried out with the participation of hepatic microsomes and organic hydroperoxides (ROOH). There is also a linear relationship between the activation enthalpy of the decomposition of the substrate — cytochrome *P450* — ROOH complex and the activation entropy:

$$\Delta H^* = 18.7 + 333\,\Delta S^*$$

A comparison of the oxidation characteristics for a given substrate in systems with cofactors, NADPH and O_2, and in systems with ROOH, allowed a conclusion to be drawn as to the rate-limiting step of oxidation. Such a step may be the insertion of active oxygen into the substrate.

The results of an investigation of two types of model systems for the enzymatic hydroxylation have been discussed.

INTRODUCTION

The hydroxylating enzyme system of mammalian liver microsomes is responsible for the metabolism of carcinogens, toxins, drugs and steroid hormones. It is a multicomponent system composed of NADPH, NADPH-cytochrome *P450*-reductase, an unidentified electron carrier, X, cytochrome *P450* and molecular oxygen[1,2]. NADPH-cytochrome *P450*-redutase is a flavoprotein of molecular weight 40 550, containing 1 mole of flavin per one subunit. Component X may be a fraction of cytochrome b_5[2]. The terminal oxidase, cytochrome *P450,* is a hemoprotein, molecular weight 45 000, containing 1 heme group per 1 mole of the enzyme[1].

* Presented by D. I. M. at the Scientific Conference »*Cytochrome P450 — Structural Aspects*« (held in Primošten — Yugoslavia, 6—10. October, 1976).

The process of substrate hydroxylation is a complex one. It is composed of 5 stages shown in Figure 1[1,2]. The oxidized cytochrome *P450* coupled with a substrate in a complex is reduced by one electron from the transport chain specific for NADPH. The reduced enzyme-substrate complex of Fe^{2+} associates rapidly with an oxygen molecule; a ternary substrate-cytochrome *P450* $-O_2$ complex is reduced by another electron from the transport chain specific for NADH. Subsequently, one activated oxygen atom is inserted into the substrate, while another one is reduced to water.

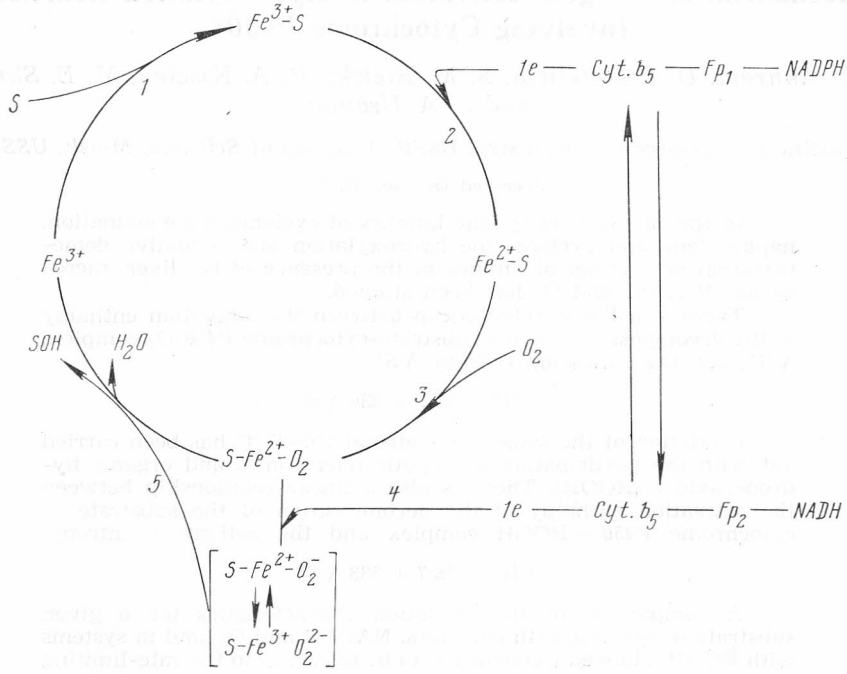


Figure 1. Scheme of hydroxylation with the participation of microsomal cytochrome *P450*.

The enzyme system of liver microsomes oxidizes aliphatic compounds to alcohols, aromatics — to phenols, demethylates methyl-substituted amines, epoxidizes unsaturated compounds, and deaminates amines[1,2].

RESULTS AND DISCUSSION

By the differential spectrophotometry method the spectra are registered at 20 °C following the interaction of the oxidized cytochrome *P450* of the liver microsomes with substrates of various chemical nature. Type I substrates, naphthalene, cyclohexane, 1-naphthol, phenobarbital etc., induce spectra with a maximum at 390 nm and a minimum at 420 nm due to an interaction with the hydrophobic sites of the apoenzyme. Type II substrates, piperidine, acetanilide nicotinamide and aniline are characterized by spectra with a minimum at 390 nm and a maximum at 430 nm. Acetanilide, nicotinamide and aniline give spectra with two maxima and minima which can be explained by the two forms of the heme iron. Type II substrates interact directly with heme

iron at the expense of unpaired electrons on the nitrogen atom. The dissociation constants, K_s, of the enzyme substrate complex are calculated from spectral parameters. They are presented in Table I. According to Table I the most stable complexes with cytochrome *P450* are formed by piperidine, 1-naphthol, cyclohexane and naphthalene[3,4].

It is of interest to note the temperature dependence of the K_s constants. In the 20—37 °C temperature range for a type I substrate, 1,2,7-trimethyldecahydroquonolone-4 (quinolone), the value of $K_s = 4.14 \exp(-625/RT)$ mol/l has been obtained, while for a type II substrate, monomethylaniline, $K_s = 10^8 \exp(6500/RT)$ mol/l. The association of quinolone with cytochrome *P450* decreases with temperature whereas that of methylaniline increases. From the association constants of the substrates with cytochrome *P450*, ΔS_o values of +36.6 cal mol^{-1} K^{-1} and —2.83 cal mol^{-1} K^{-1} were calculated for monomethylaniline and quionolene, respectively.

TABLE I

Spectral parameters for the interaction of cytochrome P450 with substrates at 20 °C [3,4]

Substrate	Protein mg/ml	Cyt. *P450* nmol/mg	λ max nm	λ min nm	$10^4 \cdot K_s$ M
Naphthalene	3.0	0.58	393	424	0.67
Cyclohexane	2.0	0.47	391	417	0.49
1-Naphthol	3.8	0.58	392	420	0.19
Phenobarbital	4.0	0.44	391	423	-
Aminopyrine	3.0	0.50	390	421	3.38
Dimethylaniline	3.0	0.50	390	421	4.65
Piperidine	3.5	0.69	427	389	0.34
Aniline	4.0	0.44	429	385	
			440	397	1.60
Acetanilide	4.0	0.44	426	385	
			440	398	3.30
N-methylaniline	3.0	0.50	421	383	7.10
Nicotinamide	4.0	0.44	426	386	
			440	398	10.00

The different temperature dependence of the K_s constant for type I and II substrates reflects the difference in the nature of their binding to cytochrome *P450*.

In the 20—37 °C temperature range using a buffer solution a comprehensive study has been made of the kinetics of naphthalene oxidation to 1-naphthol, and cyclohexane to cyclohexanol along with the kinetics of oxidative amine demethylation and cyclohexene epoxidation. These reactions proceed with the participation of cytochrome *P450* and cofactors, NADPH and molecular oxygen. With all the substrates oxidation was achieved at saturation in cofactors, NADPH and O_2, and can be described using the Michaelis-Menten equation. Michaelis constants and the maximal reaction rates were determined by the Lineweaver-Burk method. The rate constant of the decomposition of the enzyme-substrate complex can be calculated from the maximal rate values, assuming the oxygen atom insertion into the substrate in the ternary complex (substrate-cytochrome *P450*-O_2) followed by quick decomposition of the complex with product formation is the limiting step. The decomposition rate constants of the enzyme-substrate complexes are presented in Table II.

Table II shows that the kinetic parameters of cyclohexene epoxidation cyclohexane, aniline and naphthalene hydroxylation and those of the oxidative demethylation of amines are determined by the nature of the substrate oxidized[4-8]. A remarkable feature of these processes is the linear relationship

TABLE II

Kinetic parameters for the oxidation of various substrates in the presence of cytochrome P450, NADPH, and O_2 [4,8]

Substrate	Preexponential factor s^{-1}	E_{act} kcal/mole	ΔH^* kcal/mole	$-\Delta S^*$ $calK^{-1}mol^{-1}$
Cyclohexene	$4.0 \cdot 10^7$	9.80	9.30	24.2
Cyclohexane	$9.4 \cdot 10^6$	10.50	9.90	29.6
Aniline	$1.6 \cdot 10^8$	13.40	12.80	23.0
Aminopyrine	$3.6 \cdot 10^8$	13.40	12.80	21.3
Dimethylaniline	$5.6 \cdot 10^8$	13.40	12.80	20.4
Quinolone	$1.5 \cdot 10^9$	14.20	13.60	18.8
Naphthalene	$2.3 \cdot 10^9$	14.50	13.90	17.6
Monomethylaniline (demethylation)	$1.7 \cdot 10^{10}$	16.60	16.00	13.7
Naphthalene (induction MeChol.)	$4.3 \cdot 10^6$	11.00	10.40	30.0

between the enthalpy and entropy parameters (Figure 2): $\Delta H^* = A + \beta \cdot \Delta S^*$, where $A = 20.7$ kcal/mol, $\beta = 366$ K. Coefficient $\alpha = \beta/T_{avcrage} = 366/301.5 = 1.21$. Thus, the increase in activation enthalpy caused by an alteration in the chemical structure of the substrate is followed by a decrease of activation entropy (a compensation effect)[9].

It is noteworthy that the transformation of all the substrates listed in Table II proceeds with the participation of the oxidized cytochrome *P450* and organic hydroperoxides, tertbutylhydroperoxide (TBHP) and cumyl hydroperoxide (CHP). The oxidation of most compounds shown in Table II with participation of hydroperoxides and cytochrome *P450* from rat liver microsomes was studied at 20—37 °C. The kinetics of these processes at saturation of hydroperoxides is described by the Michaelis-Menten equation. The Michaelis constants and the maximal reaction rates were derived. On the assumption that the process is limited by the insertion of the oxygen into the substrate in the substrate-cytochrome *P450*-ROOH complex, the decomposition rate constants for these ternary complexes were calculated, with $E_{act.}$, ΔH^* and ΔS^* (see Table III).

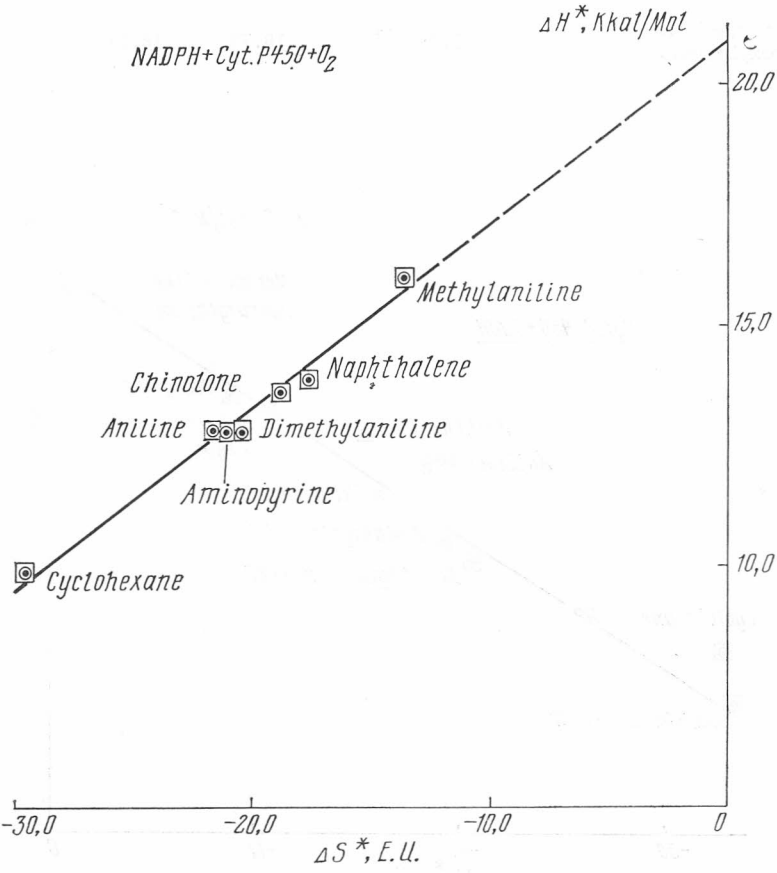


Figure 2. ΔH^* *vs.* ΔS^* plot for NADPH - cytochrome *P450* - O_2 - systems.

TABLE III

Kinetic parameters for the oxidation of various substrates involving cytochrome P450 and organic hydroperoxides[8,10-13]

Substrate	ROOH	Preexponential factor s^{-1}	E_{act} kcal/mole	ΔH^* kcal/mole	$-\Delta S^*$ $calK^{-1}mo$
Cyclohexene	TBHP	$8.6 \cdot 10^5$	8.15	7.55	33.2
Cyclohexane	TBHP	$1.0 \cdot 10^6$	10.00	9.40	33.0
Dimethylaniline	CHP	$5.1 \cdot 10^8$	12.20	11.60	20.6
Aminopyrine	CHP	$1.1 \cdot 10^9$	12.70	12.10	19.2
Quinolone	CHP	$6.4 \cdot 10^9$	13.70	13.10	16.3
Dimethylaniline	TBHP	$7.8 \cdot 10^{10}$	15.80	15.20	10.6
Monomethylaniline (demethylation)	CHP	$2.0 \cdot 10^{11}$	16.10	15.50	8.8
Monomethylaniline (hydroxylation)	CHP	$1.3 \cdot 10^{13}$	18.70	18.10	0.5

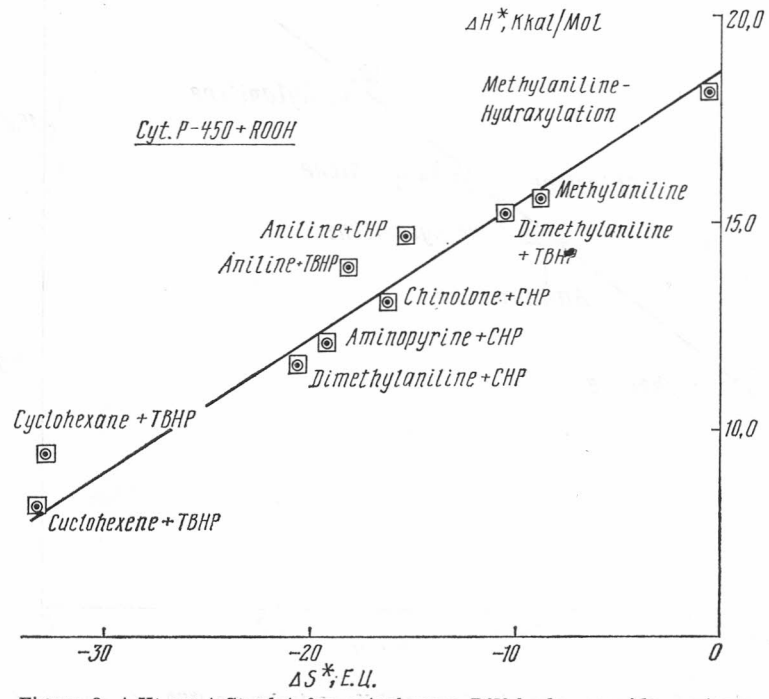


Figure 3. ΔH^* *vs* ΔS^* plot for cytochrome *P450*-hydroperoxides-systems.

A comparison between Tables II and III shows that the activation energy of oxidation for the same substrate in the system with cofactors and in the systems with hydroperoxides is either identical or very similar. The coefficients of the compensation relationship for both systems are also similar:

System	A, kcal/mol	β, K	$\alpha = \beta/T_{aver.}$
NADPH - cyt *P450* - O_2	20.7	366	1.21
cyt *P450* - ROOH	18.7	333	1.11

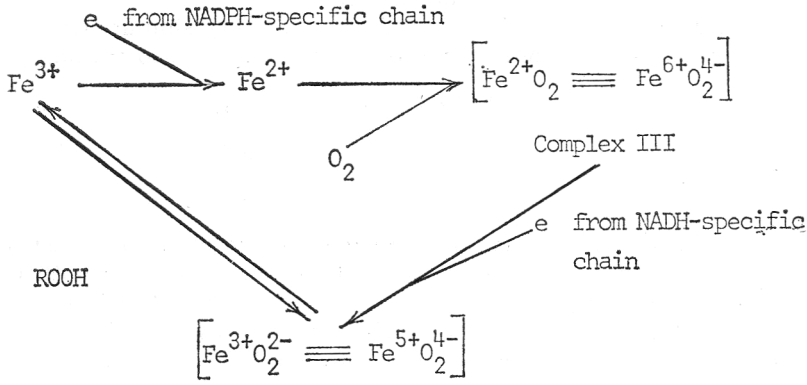
The similarity of kinetic and thermodynamic data obtained for both systems indicates that the same step can limit the process in both cases. We consider the act of oxygen insertion into a substrate of the ternary complex to be such a step. The conclusion is strengthened by the effect of the substrate on the energy characteristics of oxidation[5–8,10–13].

It is a known fact that substrate interaction with cytochrome *P450* and the addition of oxygen to the substrate-cytochrome *P450* complex are very rapid[1–3]. In the process of oxidation either reduction of the ternary complex by the second electron, or oxygen incorporation into the substrate may be the rate-limiting step. In systems with hydroperoxides there is no necessity of reduction by the second electron since the oxygen of hydroperoxide is already reduced by the two electrons. This suggests that in the cytochrome *P450*-ROOH systems the process can be limited by oxygen instertion into the substrate. The energy characteristics of processes involving the cytochrome *P450*-ROOH and the NADPH-cytochrome *P450*-O_2 systems are shown to be similar or identical for each particular substrate (see Tables II and III). Thus, in both cases the activation energies are associated with the same act, *i. e.* oxygen insertion into the substrate. This interpretation is supported by the data on primary isotopic effects during microsomal oxidation of aromatic compounds[14] or saturated compounds[15], demethylation of 4-nitroanisole[16], and ω-oxidation of natural saturated fatty acids[17]. The participation of the C-H bonds of the oxidized substrates in the limiting step is substantiated by the existence of »decoupling« compounds, such as fluorinated hydrocarbons, which interact with cytochrome *P450,* but are not oxidized since the stable C-F bond cannot be broken[18–21]. There is often no direct correlation between the rate of hydroxylation and the activity of the NADPH-cytochrome *P450* reductase. It has also been shown that in aniline hydroxylation and ethylmorphine oxidation the reduction of the cytochrome *P450*-substrate complex is not a limiting step[22,23].

Our data and the facts mentioned earlier suggest that the process of hydroxylation is limited by oxygen insertion into the substrate with subsequent quick decomposition of the ternary complex. This is supported by the remarkable work of Coon and coworkers[24], who showed that in the presence of three substrates, benzphetamine, cyclohexane and *p*-nitroanisole, the oxidation of cytochrome *P450* in the complex with oxygen is the slowest step.

The nature of the hydroxylating agent is of great interest. In the scheme below the process of oxygen activation in systems with hydroperoxides and in the NADPH-cytochrome *P450*-O_2 system is shown.

SCHEME 1.



Complex I

Complex I, well known in enzymology, acts as a hydroxylating agent. It is formed in the NADPH-cytochrome *P450*-O_2 systems *via* a successive reduction of the Fe^{3+} of cytochrome *P450* to $Fe^{3+}O_2^{2-}$ by two electrons. In Complex I the electrons may have various distributions among the atoms of iron and oxygen. The iron of this Complex is known to be in the oxidation state 5 [25]. The existence of high valency states of iron is evidenced by the ESR spectra for the systems »microsomes + ROOH« and »H_2O_2 + metmyoglobin«[26]. In the cytochrome *P450*-ROOH system, as shown in Scheme 2, Complex I exists in an equilibrium reaction:

$$Fe^{3+} + ROOH \rightleftharpoons [FeOOR]^{2+} + H^+.$$

Complex I may be the agent acting both in the system NADPH-cytochrome *P450*-O_2 and in the cytochrome *P450*-ROOH systems.

It is known that Complex I is formed during the interaction of peroxidases with hydrogen peroxide. Cytochrome *P450* shows peroxidative activity towards NADH, NADPH and tetraphenyl-*p*-phenyldiamine[27,28]. In this case the following reactions occur:

$$[Fe^{3+}O_2^{2-} \equiv Fe^{5+}O_2^{4-}] \xrightarrow{e} [Fe^{3+}O_2^{3-} \equiv Fe^{4+}O_2^{4-}] \xrightarrow{e} Fe^{3+}$$

Complex I Complex II

Complex I obtains one electron from the substrate and is converted to Complex II, which upon obtaining another electron is reconverted to the initial form, Fe^{+3}.

The oxygen transport from Complex I to the substrate may follow an oxenoid mechanism: in this case Complex I obtains two electrons at a time from the substrate molecule. Such a process is likely to occur during the oxidation of various compounds in the NADPH-cytochrome *P450*-O_2 and cytochrome *P450*-ROOH systems. In peroxidase reactions Complex I does not obtain two electrons at a time but one by one to form an intermediate Complex II, detected by ESR.

Studies of the kinetics and mechanism of reactions in systems which, to a certain extent, imitate the oxygen transfer to the substrate *via* enzyme systems, may be very important in establishing the mechanism of molecular

oxygen activation by the monooxygenase enzymes. The enzymatic hydroxylation may be imitated by two types of systems[29]:

1) the first group of systems includes an ion or a metal complex, a redox-active ligand and molecular oxygen;

2) the second group includes peracids and peroxocomplexes of molybdenum and other metals, where oxygen is already activated by the reduction with two electrons.

In our laboratory a systematic study of hydroxylation reactions involving systems of both types has been carried out[29].

In Table IV the first type of model systems is presented.

TABLE IV

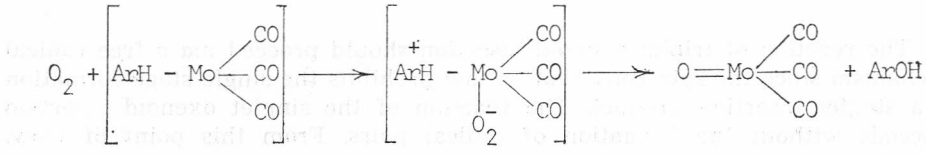
Models with molecular oxygen

System	Solvent	Substrate	Product	Reference
$Mo(CO)_6 - O_2$	Acetonitrile Benzonitrile	ArH	ArOH	29-31
$SnCl_2 - O_2$	Acetone	C_6H_{12}	$C_6H_{11}OH$	32
$FeCl_2 - O_2$	Methanol	C_6H_{12}	$C_6H_{11}OH$	33
$TiCl_4$ - NADH - O_2	$H_2O + CH_3CN$	ArH	ArOH	34
$CuCl_2$-pyrocatechol - O_2	H_2O + dioxane	ArH	ArOH	35
NADH-riboflavin -O_2- $FeCl_3$	phosphate buffer, pH = 7.4	ArH	ArOH	36

In an aqueous medium (or protonic solvent) the radicals $HO_2^{\cdot}$ and $HO^{\cdot}$ interact with the substrate by the usual chain mechanism and function as hydroxylating agents. These radicals are formed during the oxidation of an ion or a metal complex by molecular oxygen. In an aprotonic medium metal-oxygen complexes which transport the oxygen atom to the substrate by an oxenoid mechanisms, act as hydroxylating agents.

An interesting example imitating »internal« monooxygenases is the oxidation of aromatic compounds by $Mo(CO)_6$ and O_2.

It is not only oxygen which is activated on the molybdenum atom, but the aromatic substrate as well, *i. e.* there is one centre for activation of O_2 and the substrate:



In Table V the second type of model systems is presented. They epoxidate unsaturated compounds, cholesterol and allyl alcohol, and hydroxylate aromatic compounds, naphthalene, anthracene, pyrene and phenanthrene.

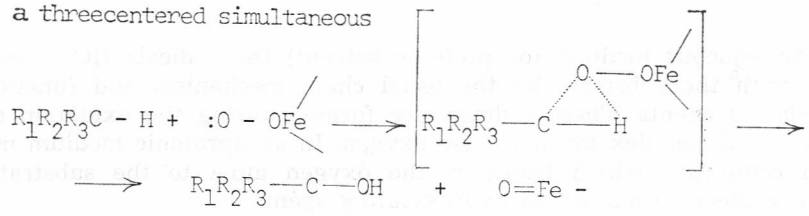
TABLE V

Model systems of the peroxide type

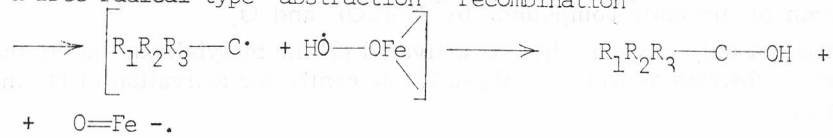
System	Substrate	Product	Reference
Peracids: HCO_3H, CH_3CO_3H, CF_3CO_3H, $C_6H_5CO_3H$	ArH	ArOH	37-39
$MoO(O_2)_2$ HMPTA	Cholesterol	5,6-epoxide cholesterol	40
$MoO(O_2)_2$ HMPTA	Allylalcohol	Glicidole	41
$MoO(O_2)_2$ HMPTA	Naphthalene	1-naphthol	42,43

It appears important to discuss the mechanism of oxygen insertion into the substrate with the participation of enzyme and model systems. Cytochrome *P450,* to be more strict, Complex I and the first group of models in aprotonic media, transport the oxygen atom *via* an oxenoid mechanism. As in the reactions of carbenes and nitrenes two ways of oxygen insertion into an ordinary C-H bond can be proposed:

a threecentered simultaneous



a free radical type "abstraction - recombination"



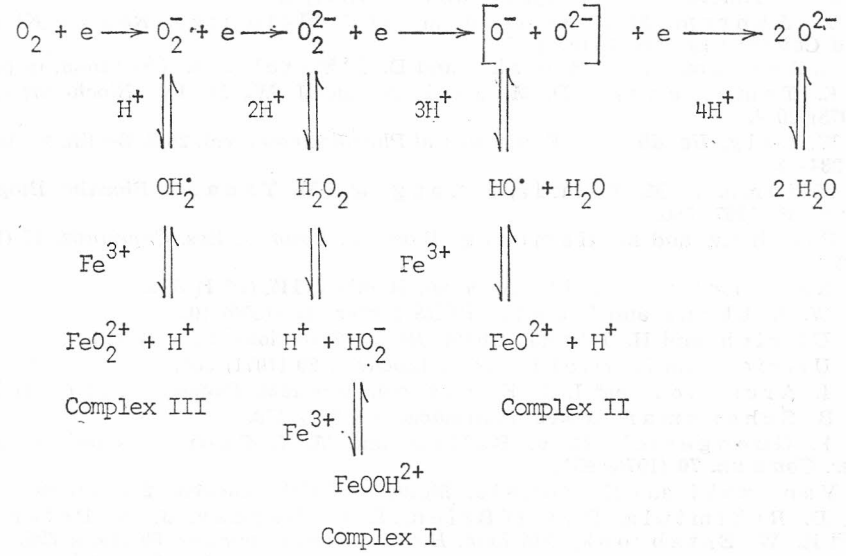
+ O=Fe -.

The reaction of triplet oxenoid insertion should proceed *via* a free radical mechanism since the spin conservation law prohibits the single stage formation of a singlet insertion product. The reaction of the singlet oxenoid insertion proceeds without the formation of radical pairs. From this point of view,

peracids, peroxy-complexes of molybdenum and Complex I in hydroxylation reactions represent typical singlet oxenoids. The ion complexes of iron and tin in aprotonic media should be triplet oxenoid ones and therefore react by the formation of radical pairs. The effect of radical reaction inhibitors does not allow one to distinguisch between a radical and a triplet oxenoid[29,33].

A comprehensive study of all the possible mechanisms for hydroxylation[29,44,45] showed that in radiation and chemical systems $HO^{\cdot}$ and $HO_2^{\cdot}$ radicals may function as hydroxylating particles, whereas in enzyme and model systems this function is fulfilled by the ion complexes of iron and oxygen. Various mechanisms are frequently opposed to one another though there is often a close relation between them. We shall show such a relation by successive consideration of oxygen reduction and the fate of intermediate particles in the presence of iron ions (see Scheme 2).

SCHEME 2.



Scheme 2 reveals that in the presence of iron ions the radicals formed in various stages of the oxygen reduction may be stabilized by ions resulting from Complexes I, II and III discussed earlier. Thus, depending on the conditions of oxygen reduction, $HO_2^{\cdot}$ or $HO^{\cdot}$ radicals or their stabilized modifications, complexes I, II and III may function as hydroxylating agents. The nature of a hydroxylating agent is primarily defined by the presence of hydrogen ions: in aprotonic media the perferrylion (Complex III), while in protonic media $HO_2^{\cdot}$ and $HO^{\cdot}$ radicals function as hydroxylating species.

A simultaneous study of the kinetics and mechanism of enzyme and model systems has led to the synthesis of simple chemical systems imitating the action of enzyme systems. So far, model systems cannot compete with the enzymes.

However, they are useful in studying the mechanism of oxygen activation and establishing conditions under which inert substrates are converted to oxyderivatives in a manner analogous to that achieved by monooxynases.

REFERENCES

1. G. S. Boyd and R. M. S. Smellie, Eds., *Biological Hydroxylation Mechanisms,* Academic Press, London-New York, 1972.
2. A. I. Archakov, *Microsomalnoe okislenie,* Nauka, Moskva, 1975.
3. A. A. Akhrem, S. S. Dvornikov, S. A. Usanov, and D. I. Metelitza, *Izvestia AN BSSR, seria chim. nauk* **5** (1976) 93.
4. A. A. Akhrem, S. M. Bielski, and D. I. Metelitza, *Biochimia,* in press.
5. A. A. Akhrem, M. E. Skurko, S. S. Dvornikov, and D. I. Metelitza, *Doklady AN SSSR* **223** (1975) 1130.
6. A. A. Akhrem, S. A. Usanov, S. S. Dvornikov, and D. I. Metelitza, *Doklady AN SSSR* **223** (1975) 1014.
7. A. A. Akhrem, S. M. Bielski, and D. I. Metelitza, *Biochimia,* in press.
8. A. A. Akhrem, T. A. Timoshchuk, and D. I. Metelitza, *Eur. J. Biochem.,* submitted for publication.
9. R. Lumry and S. Rajender, *Biopolymers* **9** (1970) 1125.
10. A. A. Akhrem, M. L. Abeliovich, S. S. Dvornikov, S. A. Usanov, and D. I. Metelitza, *Bioorganicheskaja Chimia* **2** (1976) 549.
11. A. A. Akhrem, S. A. Usanov, S. S. Dvornikov, and D. I. Metelitza, *Reaction Kinetics and Catalysis Letters,* **6** (1977) 2.
12. A. A. Akhrem, M. E. Skurko, and D. I. Metelitza, *Reaction Kinetics and Catalysis Letters* **4** (1976) 25.
13. A. A. Akhrem, S. M. Bielski, and D. I. Metelitza, *Biochimia,* in press.
14. J. E. Tomaszewski, D. M. Jerina, and J. W. Daly, *Biochemistry* **14** (1975) 2024.
15. J. W. Daly, *Handbook of Experimental Pharmacology,* vol. **28/2,** Berlin, Springer, p. 284—311.
16. C. Mitoma, D. M. Yasuda, J. Tagg, and M. Tanabe, *Biochim. Biophys. Acta* **136** (1967) 566.
17. J. Björhem and M. Hamberg, *Biochem. Biophys. Res. Communs.* **47** (1972) 333.
18. S. Narasimhulu, *Archiv. Biochem. Biophys.* **147** (1971) 384.
19. D. W. Ribbons and I. Onta, *FEBS Letters* **12** (1970) 105.
20. V. Ullrich and H. Diehl, *Chem. Biol. Interactions* **3** (1971) 310.
21. V. Ullrich and H. Diehl, *Eur. J. Biochem.* **20** (1971) 509.
22. A. I. Archakov and I. I. Karuzina, *Biochem. Pharmacol.* **23** (1974) 1053.
23. J. B. Schenkman, *Molec. Pharmacol.* **8** (1972) 178.
24. F. P. Guengerich, D. P. Ballou, and M. J. Coon, *Biochem. Biophys. Res. Commun.* **70** (1976) 951.
25. I. Yamazaki and K. Yokota, *Molec. and Cell Biochem.* **2** (1973) 39.
26. A. D. Rahimtula, P. J. O'Brien, E. G. Hrycay, J. A. Peterson, and R. W. Estabrook, *Biochem. Biophys. Res. Communs.* **60** (1974) 695.
27. E. G. Hrycay and P. J. O'Brien, *Archiv. Biochem. Biophys.* **153** (1972) 480.
28. W. R. Bidluck and P. Hochstein, *Life Science* **14** (1974) 2003.
29. A. A. Akhrem, D. I. Metelitza, and M. E. Skurko, *Uspechi Chimii* **44** (1975) 868.
30. A. A. Akhrem, M. L. Abeliovich, and D. I. Metelitza, *Doklady AN SSSR* **213** (1973) 1079.
31. A. A. Akhrem, M. L. Abeliovich, S. N. Kovalenko, and D. I. Metelitza, *Neftechimia* **14** (1974) 746.
32. A. A. Akhrem, M. E. Skurko, D. I. Metelitza, and S. M. Bielski, *Kinetika i kataliz* **16** (1975) 366.
33. A. A. Akhrem, D. I. Metelitza, and M. E. Skurko, *Bioorganic Chem.* **4** (1975) 307.
34. A. A. Akhrem, P. A. Kiselev, and D. I. Metelitza, *Kinetika i kataliz,* **18** (1977) in press.
35. A. A. Akhrem, P. A. Kiselev, and D. I. Metelitza, *Kinetika i kataliz* **17** (1976) 1492.
36. A. A. Akhrem, S. Yu. German, and D. I. Metelitza, *Doklady AN SSSR,* in press.

37. A. A. Akhrem, P. A. Kiselev, and D. I. Metelitza, *Kinetika i kataliz* **16** (1975) 1400.
38. A. A. Akhrem, P. A. Kiselev, and D. I. Metelitza, *Doklady AN SSSR*, **220** (1975) 593.
39. A. A. Akhrem, P. A. Kiselev, and D. I. Metelitza, *Reaction Kinetics and Catalysis Letters* **2** (1975) 177.
40. A. A. Akhrem, T. A. Timoshchuk, and D. I. Metelitza, *Doklady AN SSSR* **219** (1974) 891.
41. A. A. Akhrem, T. A. Timoshchuk, and D. I. Metelitza, *Tetrahedron* **30** (1974) 3165.
42. A. A. Akhrem, S. N. Kovalenko, and D. I. Metelitza, *Doklady AN SSSR* **216** (1974) 830.
43. A. A. Akhrem, S. N. Kiseleva, P. A. Kiselev, and D. I. Metelitza, *Reaction Kinetics and Catalysis Letters* **2** (1975) 375.
44. D. I. Metelitza, *Uspechi chimii* **40** (1971) 1175.
45. D. I. Metelitza, *Uspechi chimii* **41** (1972) 1737.

DISCUSSION

M. J. Coon:

The results you obtained with organic hydroperoxides are particulary interesting. Since you have mentioned free radicals, I would like to ask whether
(*a*) You have used free radical-trapping agents to examine the mechanism of the peroxide-dependent reactions?
(*b*) Do different peroxides give different results with the same substrate?
(*c*) Have you observed heme destruction due to the presence of peroxides?

D. I. Metelitza:

(*a*) Yes, we used free-radical inhibitors — α-naphtol and tritert. butylphenol — to examine the reaction mechanisms. The hydroxylation mechanism in systems »microsomes-ROOH« is an oxenoid one, but it is likely that the radicals are liberated during this process.
(*b*) Different peroxides give various reaction rates with the same substrate. The reaction rate for a given peroxide using different substrates depends on the substrate nature.
(*c*) With cumene peroxide we observed heme destruction, but to a lesser degree with butyl hydroperoxide.

F. Jung:

Do you think that the compensation relatioship has some special meaning?

D. I. Metelitza:

The comparison of the coefficients for the two compensation relationships are of special significance, and they are similar for both systems, namely with hydroperoxides and with the cofactors — NADPH and O_2. This suggests a common process.

SAŽETAK

Mehanizam aktivacije kisika u reakcijama hidroksilacije citokromom *P450*

A. A. Akhrem, D. I. Metelitza, S. M. Bielski, P. A. Kiselev, M. E. Skurko i S. A. Usanov

Ispitivana je kinetika epoksidacije cikloheksena, hidroksilacije naftalena i cikloheksana, te oksidativne demetilacije grupe amina, mikrosomima iz jetre s NADPH i O_2, u intervalu 20—37 °C. Nađena je kompenzacijska zavisnost između entalpije i entropije aktivacije ($\Delta H^* = A + \beta \Delta S^*$). Vrijednosti $A = 20.7$ kcal i $\beta = 366$ K, tako dobivene, vrlo su slične onima kada se mikrosomima jetre dodaju samo organski hidroperoksidi (ROOH): $A = 18.7$ kcal i $\beta = 333$ K. Zaključuje se da je najsporija faza oksidacije ugrađivanje aktiviranog atoma kisika u supstrat ternarnog kompleksa. Opisani su i rezultati dvaju modelnih sistema s metalnim ionima, te uloga radikala u pojedinim fazama reakcije.

INSTITUTE OF BIOORGANIC CHEMISTRY
BSSR ACADEMY OF SCIENCES, MINSK, USSR

Prispjelo 12. listopada 1976.