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Biomimetic alkane oxidation involving metal complexes

E I Karasevich, V S Kulikova, A E Shilov, A A Shteinman

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Abstract. The state-of-the-art of investigations in the field of catalysis of biological oxidation of saturated hydrocarbons by molecular oxygen and simulation of this process based on metal complexes is reviewed. The bibliography includes 296 references.

I. Introduction

Functionalisation of alkanes is of both theoretical and practical interest. Progress in homogeneous catalysis brought forth the problem of activation of C–H bonds as long as several decades ago. However, despite the considerable achievements in this area [1] practical requirements have stimulated the search for novel reactions and elaboration on their basis of more efficient procedures for alkane conversions. Currently, this is particularly urgent when the scantiness of natural resources of this valuable chemical raw material has become obvious. Petroleum and natural gas, the main sources of alkanes, are now mostly used as a fuel. Of nearly a billion tons of the petroleum extracted in the world, only 5% is chemically processed and direct functionalisation of alkanes is as low as fractions of a per cent. The inertness of alkanes is the main obstacle to the development of methods for their functionalisation; reactions with these compounds occur under drastic conditions and with low selectivity.

The main pathway of alkane functionalisation is their oxidation. In the living nature, this problem has been successfully solved in two ways: through aerobic oxidation, which involves molecular oxygen, and an anaerobic one. The corresponding natural enzymic systems still remain unsurpassed as regards the rate and the selectivity of alkane oxidation. Therefore, the biomimetic approach, *i.e.*, creation of chemical analogues of enzymes, seems to be especially promising in this respect. The current views about the aerobic biological oxidation of alkanes involving monooxygenases and its chemical simulation are discussed in this review.

E I Karasevich N M Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, ul. Kosygina 4, 117977 Moscow, Russian Federation. Fax (7-095) 137 41 01. Tel. (7-095) 939 72 43
V S Kulikova, A E Shilov, A A Shteinman Institute of Chemical Physics in Chernogolovka, Russian Academy of Sciences, 142432 Chernogolovka, Moscow Region, Russian Federation. Fax (7-096) 515 35 88.
Tel. (7-095) 542 50 35 (V S Kulikova), (7-095) 524 50 35 (A E Shilov)

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II. Monooxygenases

The term 'monooxygenases' is generally applied to the enzymes that catalyse the oxidation of a substrate by molecular oxygen in the presence of a reducing agent according to the stoiochiometric equation:

$$RH + O_2 + 2H^+ + 2\bar{e} \longrightarrow ROH + H_2O.$$

Thus, one atom of the oxygen molecule is incorporated into the substrate, whereas the other one forms water. Many monooxygenases contain metals as constituents, in the first place, iron and copper. Two equivalents of a reducing agent, which is needed for the monooxygenase cycle to be accomplished, come from an organic component that must be always present in the system. The role of this compound is to transfer electrons to the metal atom of the enzyme, which passes into the reduced state and thereby acquires the ability to react with molecular oxygen and activate it for further interactions with alkanes.

Hence, according to the current concepts,[2] activation of molecular oxygen resulting in the formation of metal peroxo- and oxo-complexes is the basic mechanism of the catalytic effect of monooxygenases. The chemistry of these complexes and their reactions with alkanes have attracted keen attention of investigators in the past decade.

1. Heme monooxygenases

a. Cytochrome P-450

Among monooxygenases, the suprafamily of cytochrome P-450 has been known for the longest time.[3–5] Its active centre, like hemoglobin or myoglobin, contains heme, an iron-protoporphyrin complex. These enzymic systems can oxidise hundreds of thousands of low-molecular-weight compounds of both endogenous and exogenous origin.[6–8] Cytochrome P-450 catalyses hydroxylation of alkanes, epoxidation of alkenes, *O*- and *N*-dealkylation, and other oxidation reactions. About 500 representatives of the cytochrome P-450 suprafamily are currently known.[7,9] They differ from one another in the structure of their globular protein in the region of the active centre, but have identical prosthetic groups.

Cytochrome P-450 is the component of a multienzyme system, which also contains electron transfer enzymes. In mitochondria and most bacteria, electrons are transferred from NADH and NADPH to the enzyme by FAD-dependent reductase *via* iron–sulfur ferredoxin. In systems of the microsomal endoplasmic reticulum devoid of Fe_2S_2-ferredoxin, the electrons are transferred

from NADPH by cytochrome P-450 oxidoreductase, a flavoprotein that contains FAD and FMN.

The catalytic cycle of cytochrome P-450 varies depending on the electron source and the type of substrate being metabolised; however, its main stages are common for the whole cytochrome P-450 suprafamily (Fig. 1). The further the stage from the original state Fe^{III} in the catalytic cycle the less studied the structure of the intermediates and the mechanism of their formation. The reason is that the time of conversion of the corresponding intermediate may be too small for the latter to be registered by the modern technique.

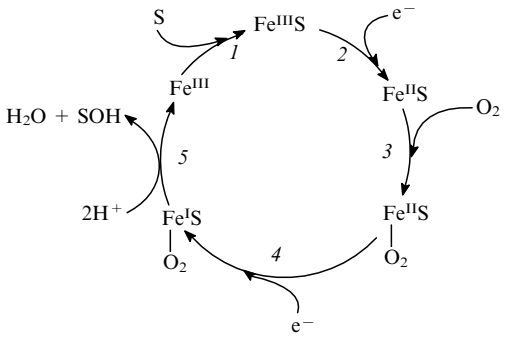


Figure 1. The catalytic cycle of cytochrome P-450.

The kinetics of individual stages of the catalytic cycle has been studied for both bacterial [10] and microsomal [7] cytochrome P-450. The system of camphor monooxygenation from *Pseudomonas putida*, cytochrome $P\text{-}450_{cam}$ (CYP101),[11] is being studied in more detail in comparison with microsomal cytochrome P-450 membrane-bound proteins the analysis of which in the isolated state faces certain difficulties. Interaction with the phospholipid bilayer can influence the generation of a catalytically active conformation of membrane-bound enzymes. However, kinetic regularities of these systems have much in common [12] (Table 1), which makes it possible to propose a unified mechanism for oxygen activation in liver and bacterial cytochrome P-450.

Table 1. The rate constants for various stages of the catalytic cycle of cytochrome P-450 in bacterial and microsomal systems.[7, 12]

Stage [a]	Cytochrome $P\text{-}450_{cam}$ [b]		Microsomal cytochrome P-450 [c]
	T /°C	k	k
1	4	$47\ s^{-1}$	$50\ s^{-1}$
2	25	$30-33\ s^{-1}$	$18\ min^{-1}$
			$11.5\ min^{-1}$ (see [d])
3	20	$1.7 \times 10^6\ M^{-1}\ s^{-1}$	$> 10^6\ M^{-1}\ s^{-1}$ (see [e])
4		$1-4\ s^{-1}$	$2-7\ s^{-1}$

[a] See Fig. 1; [b] turnover number is $1900\ min^{-1}$; [c] turnover number is $24\ min^{-1}$ (benzphetamine, cytochrome $P\text{-}450_{LM2}$); [d] without substrate; [e] 25 °C.

Numerous spectral studies [7, 12, 13] have established, with a sufficient degree of reliability, the nature of the intermediate complexes formed in the catalytic cycle of cytochrome P-450, starting from the original Fe^{III} to the $Fe^{II}O_2$-complex. An equilibrium between the low-spin and the high-spin states of Fe^{III} in the original enzyme is shifted towards the former owing to the presence of a water molecule in a *trans*-position relative to the thiolate.[14] Hydrophobic binding of a substrate results in the dissociation of the ligand from the 6th position and a shift of the equilibrium towards the high-spin state ($S = 5/2$). This is accompanied by a simultaneous increase in the redox potential of the iron complex, which facilitates electron transfer from the reductase to yield a high-spin Fe^{II}-heme ($S = 2$). Interaction of the latter with O_2 gives an oxygen complex similar to that of hemoglobin. At lower temperatures, this complex is relatively stable and can be identified by spectral methods for various isoforms of cytochrome P-450.[15, 16]

Transfer of the second electron to oxycytochrome P-450 results in the formation of $Fe^{2+}O_2^{2-}$ or $Fe^{3+}O_2^{2-}$ species. In the absence of the substrate,[12] decomposition of these species yields a superoxide, the rate constant being $29\ min^{-1}$. Binding of a substrate with the enzyme inhibits this process; the rate constant for the formation of oxidation products is about $30\ min^{-1}$ and it changes slightly depending on the nature of the substrate and the enzyme.

The intermediate stages following the oxycytochrome reduction occur too fast for the intermediates to be detected by spectral methods. The mechanism of transformation of the substrate–reduced oxycytochrome complex is the least understood in the catalytic cycle of cytochrome P-450. It is usually assumed [7] that the known oxidation products are formed through heterolytic or homolytic cleavage of the O—O bond in the iron peroxo-complex. Different versions of this mechanism agree only with certain parts of the experimental data available. Presumably, the operation of a particular mechanism depends on the nature of the substrate and the enzyme.

Most of investigators postulate the pentavalent iron oxene complex ($P\text{-}450 - Fe^V = O$) as the direct oxidant of a substrate by analogy with the reactive intermediates of the catalytic cycle of peroxidase, which have the oxoferryl group ($Fe^{IV} = O$) in their active centre. However, the Mössbauer and Raman spectra of cytochrome P-450 recorded under catalytic conditions [12] suggest the presence of iron-superoxide structures similar to oxyhemoglobin HbO_2^-, but different from the ferryl species of peroxidase. Probably, the superoxide complexes are formed as intermediate products in the activation of oxygen on cytochrome P-450. Unfortunately, the iron oxene species postulated for the catalytic cycle of cytochrome P-450 have not been identified yet by any of the existing physical methods. At the same time, the oxene species in peroxidase systems are relatively stable, well-characterised intermediates.[17–19] The formation of reactive species in the conversion of hydrogen peroxide by peroxidase occurs under conditions of acid-alkaline catalysis in the imidazole fragment of histidine localised in the distal region of the enzyme heme.[18, 20] In contrast to peroxidase, thiolate is the proximal ligand of the cytochrome P-450 heme.[14, 21, 22] Presumably, the effect of the thiolate ligand and the presence of a substrate in close proximity to the reactive oxygen of cytochrome P-450 decrease the lifetime of the active species and prevent their registration.

Not only the lack of direct proofs of the existence of iron–oxene complexes of cytochrome P-450, but some other data raise doubt about the nature of the reactive species in the catalytic cycle of this enzyme. According to calculations, the $P\text{-}450 - Fe = O$ complexes must be electrophilic.[7] In practice, the reactions catalysed by this enzymic system suggest either the electrophilic or the nucleophilic character of the reactive species. The role of the nucleophile in the catalytic cycle of cytochrome P-450 can be played by iron peroxide, whereas iron oxene and iron hydroperoxide (or hydroxide) are the possible candidates for the electrophilic reactive intermediates. Electron density calculations demonstrate that the oxygen atom that is directly bound to the heme in peroxo-ferryl complexes with thiolate ligands, bears a partial positive charge, whereas the external oxygen atom bears a partial negative charge. It may thus be assumed that peroxo-complexes function as monooxygenating species. The catalytic activity of ferrocytochrome $P\text{-}450_{cam}$ with superoxide anion is additional evidence in favour of this hypothesis.[7, 23] The peroxo intermediate is regarded as a direct oxidant in the oxidation of lauric acid [24] and in the oxidative deformylation of aldehydes by aromatase,[25, 26] it is involved in reactions catalysed by NO-syn-

thase [27] and heme-oxygenase.[28] However, the formation of epoxides and *N*-oxides in these reactions is inconsistent with reactive peroxide or superoxide intermediates and attests to the involvement of oxene species.

Cytochrome P-450 catalyses substrate oxidation in the absence of molecular oxygen with iodosobenzene, peroxides, peroxy acids, and other oxygen donors as oxidants.[7] In these so-called by-pass systems, cytochrome P-450 transfers the oxygen that is already activated from the oxidant to the substrate. In other words, it functions either as an oxene transferase or it oxidises substrates by detaching electrons as does peroxidase. A by-pass system with iodosobenzene is considered to be the best, because the composition of its monooxygenation products is the closest to that in the complete enzyme system. It should be noted, however, that the identity of the oxidation products does not always imply the identity of the reactive species in these systems.

There is evidence [7] that the structure of reactive intermediates generated in the interaction of PhIO with cytochrome P-450 is closer to that of peroxo-species (Fe—O—O) than to that of the iron oxene (F=O). The oxygen atom of iodosobenzene in these intermediates is analogous to the external oxygen atom of iron peroxide. Moreover, the use of radiolabelled water has shown that the products of oxidation in this by-pass system contain an oxygen atom from the solvent. Therefore, some investigators suggest that iron peroxide or iron hydroperoxide are the most probable reactive intermediates of the by-pass systems.

Adrenal cortex cytochromes P-450 involved in the biosynthesis of steroid hormones are highly regioselective, apparently due to the rigid fixation of the substrate in a hydrophobic pocket near the active centre. Bacterial cytochrome P-450 involved in the hydroxylation of lauric acid catalyses alkane oxidation exclusively at the methyl group,[29,30] thereby manifesting an ω-hydroxylase activity. This points definitely to very strong steric hindrances during the approximation of the alkane molecule to the active centre. However, in the case of liver cytochromes P-450 involved in detoxication of xenobiotics and capable of oxidising a wide variety of substrates, the observed regioselectivity is apparently determined mainly by the intrinsic reactivity of the active intermediate.[31] In the ω-hydroxylation of racemic, (*R*)-, and (*S*)-1,1,1-trideuterio-2-phenylpropane by rat liver microsomes, the stereoselectivity depends on the type of cytochrome P-450 and the structure of the active centre. This suggests that in this case, too, regioselectivity is determined by the structure of the apoprotein rather than by the nature of the reactive intermediate.[32] It thus follows that, depending on the apoprotein structure, identical reactive intermediates can have different selectivity in the formation of oxidation products, while different active intermediates can have similar selectivity.

Thus the nature of monooxygenating reactive species in the catalytic cycle of cytochrome P-450 is still open to discussion. In our opinion, oxoferryl complexes in cytochrome P-450-containing monooxygenases are the reactive species that oxidise the C—H bonds of alkanes.

Studies with targeted mutations [33] have shown that polar amino acid residues in the distal part of the cytochrome P-450 heme can be involved in proton transfer to the oxygen of the peroxo-complex, thus enabling the formation of an intermediate that hydroxylates the substrate. This is confirmed by the increase in the amount of peroxide and the decrease in the rate of substrate hydroxylation when the normal course of protonation is inhibited by targeted mutagenesis of threonine.[34] Simultaneous mutagenesis of aspartic acid (Asp251) and threonine (Thr252) in cytochrome P-450$_{cam}$ has demonstrated [35] a cooperative effect of these amino acid residues that results in the cleavage of the O—O bond. Very probably, this reaction is the key stage of the mechanism of formation of the oxidation products (Fig. 2).

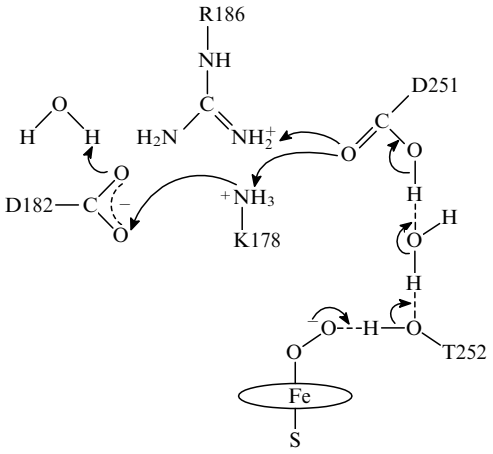


Figure 2. A schematic mechanism of the cleavage of the O—O bond in cytochrome P-450$_{cam}$.[35]

b. *N*-Methylamine monooxygenase

It is necessary to mention another heme-containing monooxygenase from *Pseudomonas asinovorans*, which catalyses *N*-demethylation of secondary amines. Like peroxidase and unlike cytochrome P-450, it contains the imidazole moiety of histidine in the 5th axial (proximal) position of the heme; however, similarly to cytochrome P-450, it oxygenates the C—H bonds of methylamines. The intramolecular kinetic isotope effect (KIE) observed [36] for the methyl group of 1,1,1′,1′-tetradeuteriodimethylamine (k_H/k_D = 1.76) is close to the KIE for the analogous reactions of cytochrome P-450 and chloroperoxidase.

2. Non-heme monooxygenases

It has been established [37–39] that non-heme iron-containing monooxygenases, such as methane monooxygenase and ω-hydroxylase, analogously to cytochrome P-450, can transfer the oxygen atom from a relatively weak oxidant, *viz*., molecular oxygen (using two reducing equivalents) or from atomic oxygen donors, such as PhIO, ClO^-, H_2O_2, RCOOOH, *etc*., to the C—H bonds of alkanes.

a. ω-Hydroxylases

ω-Hydroxylases, which contain one iron atom in their active centre, are of considerable interest as the representatives of the family of non-heme monooxygenases. They mediate targeted hydroxylation of the methyl group of n-alkyl chains of various compounds including alkanes.

The non-heme monooxygenase system of *Pseudomonas oleovorans* comprises three components that are typical of any monooxygenase system: an iron-containing monooxygenase, a reducing agent, and a carrier. In this particular case, these are ω-hydroxylase, flavoprotein reductase, and rubredoxin.[40] Together with terminal hydroxylation of the methyl group in alkanes and fatty acids, this enzymic system can epoxidise terminal alkenes, convert sulfides into sulfoxides, and demethylate ethers.[41] All these reactions are stereospecific [42] with high KIE (k_H/k_D = 6–8).[43] The Fe=O species can serve as an active intermediate in the oxidation reactions catalysed by ω-hydroxylase.[44] The use of a 'radical clock' method with *trans*-1-methyl-2-phenyl-cyclopropane as the substrate has shown [43] that 1-phenyl-but-3-en-1-ol is the only oxidation product. The conclusion on the radical attack at the C—H bond can hardly be considered as unambiguous.

Non-heme ω-hydroxylase from *Pseudomonas aeruginosa* [45] oxidises n-alkanes (C_5–C_{10}) into the corresponding acids *via* the primary alcohols. The efficiency of oxidation is lower for branched alkanes. The methyl group in 1,4-dimethylcyclohexane

is oxidised only when it occupies the equatorial position, *i.e.*, the enzyme can oxidise compounds with a relatively planar conformation. Smooth oxidation was observed for alkylbenzenes. It was thus concluded that a hydrophobic cavity (5.8 Å) exists in the vicinity of the enzyme active centre. The presence of such a cavity in this particular enzymic system as well as in other monooxygenases may account for the unusual substrate specificity and regioselectivity observed in the oxidation of hydrocarbons.

b. Methane monooxygenase

Methane is the most widespread and the least reactive representative of the family of saturated hydrocarbons. Therefore, its catalytic oxidation under mild conditions is of the greatest interest. The examples of selective low-temperature oxidation of methane are rather scarce.[37, 38, 46, 47] At the same time, the enzymic systems of various microorganisms, *viz*., methane monooxygenases (MMO), catalyse efficient conversion of methane into methanol.[48–51] Unlike heme monooxygenases and non-heme ω-hydroxylases, the iron at the active centre of MMO is a component of a binuclear centre, as in hemerythrin (HR), the protein, which is an oxygen carrier, like hemoglobin,[52–54] and in ribonucleotide reductase (RR) where the binuclear iron complex performs one-electron oxidation of tyrosine. The latter is further involved in a complex oxidation–reduction cycle.[55–58]

Several MMO from various bacterial sources, such as *Methylococcus capsulatus*,[59, 60] *Methylosinus trichosporium*,[61] and *Methylobacterium*[62] have been studied. Depending on growth conditions, the enzyme can exist both in the membrane-bound and the cytoplasmic (soluble) form.[63]

Water-soluble MMO from *M. capsulatus* has been studied in the most detail. This enzymic system oxidises the methyl group and the adjacent methylene group of n-alkanes [ω- and (ω-1)-hydroxylation]. In branched alkanes, it is the most remote from the branching part of the molecule that undergoes oxidation, which points to substantial steric hindrances in the vicinity of the active centre. Nevertheless, in the case of small molecules, *e.g.*, isobutane or *cis*-1,4-dimethylcyclohexane,[64] the oxidation affects predominantly the tertiary C–H bonds. These data suggest that the steric demands are determined not by the active centre itself, but rather by the protein environment, which forms a hydrophobic cavity of a definite size.

The MMO consist of three closely interrelated proteins, *viz*., **A**, the monooxygenase (or hydroxylase) itself, which is a dimer and contains two to four iron atoms; **B**, a regulatory protein, and **C**, a reductase.[65, 66] Protein **B** strongly affects the redox properties of the hydroxylase component of MMO.[67, 68] In the case of *M. capsulatus*, the redox potentials of hydroxylase for the transfer of the first and the second electrons are equal to +48 and −135 mV (relative to the NHE) and are slightly shifted towards more negative potentials after the addition of a substrate. In the absence of the substrate, the hydroxylase cannot be reduced even at −200 mV. The transfer of the second electron occurs more easily than that of the first one, which makes it possible to avoid the formation of the unfavourable $Fe^{III}Fe^{II}$ state of the cluster. Similarly to cytochrome P-450, binding of a substrate facilitates the reduction of iron and initiates the catalytic cycle of MMO. Binding of protein **B** results in changes in the EPR spectrum of the $Fe^{III}Fe^{III}$ form of hydroxylase and strongly influences the rate and regioselectivity of alkane oxidation. Presumably, when protein **B** binds to hydroxylase, it induces conformational changes in the region of the active centre. This in turn changes the coordination environment of the iron and, as a consequence, the spectroscopic, redox, and catalytic properties.

It was reported[69–72] that the active centre of MMO contains two iron atoms, which are linked by μ-oxo-μ-carboxylate bridges as in hemerythrin and ribonucleotide reductase.[38, 54, 57, 58] As for the terminal ligands, some indirect data show that each iron atom in the active centre of MMO is coordinated to one imidazole moiety of histidine.[73, 74]

An interesting comparison of MMO and RR can be made on the basis of X-ray data. The hydrophobic cavity in MMO is much larger than that in RR. This is consistent with the fact that MMO binds a hydrophobic substrate, *viz*., methane. The position occupied by tyrosine in RR is occupied by cysteine in MMO. This allowed one to suggest an original hypothesis[75] about the catalytic mechanism of MMO with the involvement of the SH-group. Apart from cysteine, threonine (Thr213), which is apparently necessary for proton transport in the catalytic cycle, is the only amino acid containing a protonated group vicinal to the hydrophobic cavity; its effect is similar to that of Thr252 in cytochrome P-450.[34, 35]

Thus, the spectral and X-ray data suggest that the iron in the active centre of MMO is the component of a binuclear μ-hydroxo-complex, which has much in common with the Fe–O–Fe centres of HR and RR for which X-ray data are also available (Fig. 3).[75]

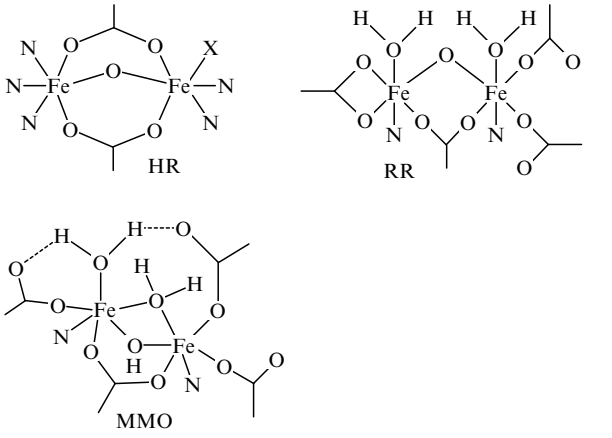
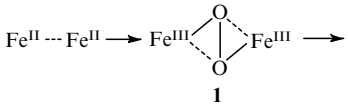


Figure 3. A schematic representation of binuclear active centres of hemerythrin, ribonucleotide reductase, and methane monooxygenase.

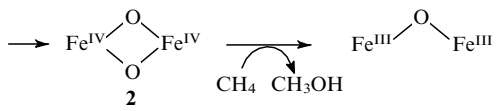
The catalytic cycle of MMO is very similar to that of cytochrome P-450. Alkane binding is followed by fast two-electron reduction of the μ-hydroxo-Fe_2^{III} to the μ-hydroxo-Fe_2^{II} state, which can rapidly react with O_2.[76] One-electron reduction gives the μ-hydroxo-$Fe^{III}Fe^{II}$ intermediate, which slowly reacts with oxygen.[48, 71]

The stepwise formation of three intermediates, *viz*., **P**, **Q**, and **T**, from μ-hydroxo-Fe_2^{II}, has been established.[50] The intermediate **P** is formed immediately after mixing of O_2 with MMO subjected to two-electron reduction; it is then slowly converted into the intermediate **Q**. The rate of conversion of the intermediate **Q** depends on the nature and the concentration of the substrate, which suggests that the intermediate **Q** is the key oxidising agent. And finally, the intermediate **T** generated upon reaction of the intermediate **Q** with the substrate dissociates to give the oxygenated product. The dissociation of the intermediate **T** is the rate-limiting step of this reaction. According to Mössbauer spectroscopic data,[49–51] the structure of the intermediate **Q** can be represented as a diferryl or a bis-μ-oxo-complex.

The binuclear structure of the active centre of MMO, the unusual substrate specificity and selectivity of alkane oxidation as well as the spectral characteristics of the reactive intermediates made it possible[77–79] to develop new concepts of the catalytic mechanism of MMO:



1



According to these concepts, activation of O_2 occurs with the formation of μ-η^2,η^2-peroxide **1** as a result of lateral binding of the O_2 molecule. This intermediate is further converted into the bis-μ-oxo-Fe_2^{IV} intermediate **2**, which reacts with methane and is an alternative to ferryl. Unlike ferryl, the O atom in this intermediate occupies the bridging position between two iron atoms and thus ensures its more efficient stabilisation.

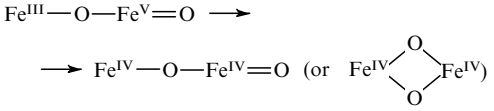
The interaction of the O atom of the intermediate **2** with the C—H bond of methane, which has the smallest length, allows an additional nucleophilic assistance of the second O-bridge, *e.g.*, due to the formation of a hydrogen bond with the H atom of methane. This speculation might rationalise the tunnelling effect of the proton in methane oxidation. Electronic and steric factors that control the interaction of the bis-μ-oxo-Fe_2^{IV} intermediate **2** with the C—H bond may account for the preferential oxidation of methane and lower alkanes and almost complete lack of selectivity in the oxidation of the bonds. Unexpectedly, the KIE for the interaction of the intermediate **Q** with CH_4 and CD_4 appeared to be very high (50–100).[80,81]

As has been noted above, mononuclear iron complexes at the active centres of various monooxygenases catalyse efficient oxidation of C—H bonds. The question of why Nature selected the binuclear iron complex as the active centre of MMO may be answered as follows: first, this allows simultaneous transfer of two electrons to the O_2 molecule thereby avoiding a thermodynamically and kinetically unfavourable stage of the formation of the oxygen radical anion en route to peroxide:

$$Fe^{II}—O—Fe^{II} \xrightarrow{O_2} Fe^{III}—O—Fe^{III}\cdot O_2^{2-}\ .$$

Second, both Fe^{III} ions can accept O^{2-} after heterolytic cleavage of the O—O bond without any preliminary protonation, which is extremely unfavourable in the strongly hydrophobic environment near the active centre.

Third, electron transfer from the neighbouring iron atom allows one to circumvent the superoxidised state of iron, which is unstable under the conditions of the non-heme environment.



In other words, the second iron atom plays the same role as the porphyrin ligand in compound I of peroxidase or in the active ferryl species of cytochrome P-450.

And finally, magnetic interaction between the iron ions of the complex enables coordination of the electron-orbital effects of both atoms in the attack at the C—H bond.

b. Toluene monooxygenases

Toluene monooxygenases T2MO, T3MO, and T4MO catalyse the formation of *o*-, *m*-, and *p*-cresols; they can also oxidise other hydrocarbons. The active centre of toluene monooxygenase comprises two iron atoms linked together by a hydroxyl bridge; its spectral characteristics are similar to those of MMO. Toluene monooxygenase catalyses the oxidation of substrates by either molecular oxygen in the presence of a reducing agent or hydrogen peroxide.[82,83]

Analysis of the data available on heme and non-heme monooxygenases demonstrates that the nature of the reactive intermediate formed in the monooxygenase-catalysed reactions is still controversial. Earlier, it was thought that the active complex of all iron-containing monooxygenases comprises the ferryl fragment — Fe=O with iron in the formal oxidation states IV and V. This concept could not be used to rationalise the experimental results obtained for the non-heme enzyme, methane monooxygenase. This problem could be surmounted when it was assumed that oxygen can bind with two iron atoms to give $Fe^{IV}(\mu\text{-}O)_2Fe^{IV}$, which can be regarded as a ferryl dimer. As far as heme systems are concerned, some investigators propose iron peroxide (or iron hydroperoxide) as the most probable reactive intermediate. Hence, a detailed structure of reactive intermediates of heme and non-heme monooxygenases has not been elucidated in full detail.

Another question of fundamental importance is the way whereby oxygen is transferred from the reactive oxidant to the hydrocarbon.

3. The mechanism of alkane monooxygenation

The nature of interactions of mono- and bi-nuclear ferryl intermediates in heme and non-heme monooxygenase systems with the C—H bonds of alkanes is still a widely debated topic.

The electronic structure of atomic oxygen is similar to that of the bivalent carbon compounds (carbenes) and by analogy it is termed oxene. Like carbenes, oxene in the triplet state reacts with alkanes as a free radical and detaches hydrogen. In the singlet state, it can also be inserted into the C—H bond to yield an alcohol.[84,85] Studies of the gas-phase reactions of singlet oxene with alkanes resulting in the generation of the OH radical showed[86] that this reaction can occur by two different routes, which are interpreted as the insertion of the O atom into the C—H bond and the detachment of the H atom. It should be noted that the insertion mechanism predominates if small molecules (CH_4, C_2H_6) with strong C—H bonds are subject to attack, whereas hydrogen detachment takes place predominantly in the reaction with higher hydrocarbons [C_3H_8, $C(CH_3)_4$] that are either sterically hindered and/or contain weaker C—H bonds. Thus the fraction of the route involving the detachment of H varies from 0.05 for CH_4 to 0.9 for $C(CH_3)_4$. It is concluded from these data[86] that the detachment can result from the intersection of two potential energy surfaces at the beginning of a single route that corresponds to insertion. The lifetime of the complex is 0.35×10^{-12} s for H_2, 0.8×10^{-12} s for CH_4, and 4.2×10^{-9} s for $C(CH_3)_4$.

The oxygen atom in the active centre of monooxygenase may be bound with the metal complex and its capacity to be inserted into the C—H bond or to detach the H atom is maintained. Such compounds (oxenoids) are related to the oxo-salts of transition metals. These compounds are also thought to be the intermediates possessing the superoxidative properties, *e.g.*, in the transfer of the oxygen atom from N_2O to metal ions.[87]

Two possible mechanisms are usually considered: (a) synchronous insertion of the O atom detached from the metal ion into the C—H bond (the concerted, oxenoid mechanism)[88–90] and (b) crypto-radical (the so-called oxygen rebound mechanism), which implies detachment of the hydrogen atom from the substrate molecule at the first stage with subsequent recombination of the alkyl radical formed with FeOH at the active centre of the enzyme.

The following data are in favour of the synchronous insertion mechanism. In the monooxygenase reaction, alkanes yield alcohols and retention of the original configuration of the asymmetric carbon atom where the oxidation involves the C—H bond is a characteristic feature in many cases.[91,92] It may therefore be assumed that this reaction proceeds without the intermediate formation of free radicals, which might lead to racemisation. Alkenes yield epoxides,[93] which retain the configuration of the original alkene.[94] Benzene and other aromatic hydrocarbons are converted into phenols and dihydrodiols.[95] Both types of compounds are formed *via* arene-1,2-oxides.[96] Migration of a group bound to the aromatic ring, which is characteristic of enzymic oxidation, from the position subject to hydroxylation to the neighbouring position (the NIH shift) is consistent with this conclusion.[97,98]. Quantum-chemical grounds for the oxenoid mechanism have been published.[99]

However, the oxygen rebound mechanism for heme and non-heme monooxygenases has recently received the most ample

recognition. This mechanism is usually supported by the following facts.

1. The large primary kinetic isotope effect in the cytochrome P-450-catalysed hydroxylation is attributed to the linear structure of the transition state (Fe—O···H—R). It is assumed that the insertion of the O atom into the C—H bond must be characterised by a small isotope effect.[100]

2. In some cases, partial or complete skeletal isomerisation of the original compound occurs en route to the reaction products. Presumably, this takes place in the intermediate alkyl radical.

3. The effect of radical traps. In the case of methane oxidation by methane monooxygenase, the use of radical traps allowed indentification of the methyl radical.[101]

Evidence for a stepwise mechanism, which involves initial detachment of the hydrogen atom by a reactive oxidising species,[102, 103] follows from the high KIE (10–12) observed in hydroxylation by cytochrome P-450-containing monooxygenases [104–107] and soluble MMO from *M. trichosporium* [108] as well as from the incomplete retention of the original configuration of the asymmetric carbon atom.

There are other facts in favour of the oxygen rebound mechanism. Thus allylic oxidation in a system with cytochrome P-450 resulted in the formation of isomeric allylic alcohols, which can be accounted for by the generation of the intermediate radical, $[C=C-CH^{\bullet} \leftrightarrow {}^{\bullet}C-C=CH]$.[109] The rate constant for the recombination of the methylcyclopropyl and OH radicals was determined, $k_{ox} \approx 2 \times 10^{10}\ s^{-1}$.[110]

Apparently, the 'radical clock' method [110] not only confirms the intermediate formation of a radical, but also permits the quantitation of its kinetic behaviour. However, further applications of this method have shown that the increase in the isomerisation rate constant (due to the incorporation of the corresponding substituent into the cyclopropane ring) does not result in a parallel increase in the yield of the isomerised product. The 'radical clock' method yielded different k_{ox} values for different molecules [from $1.4 \times 10^{10}\ s^{-1}$ to $(2-7) \times 10^{12}\ s^{-1}$] [111–113] with no obvious relation to the structure.[111–113] Moreover, the use of a supersensitive probe [114] gives an extremely high k_{ox} value of $1.4 \times 10^{13}\ s^{-1}$ (based on the yield of the isomerisation product). The lifetime of a radical with such a great value of the recombination rate constant, which also includes diffusion, must correspond to the period of one vibration.

In the case of MMO from *M. capsulatus*,[115] the rate constant for the recombination of the active oxo-species with a radical intermediate also exceeds $10^{13}\ s^{-1}$. In this case, the reaction occurred with 100% retention of configuration,[116] which obviously excludes the generation of an alkyl radical. The application of analogous tests to *M. trichosporium* has shown the formation of a small amount of rearranged products, which might attest to the presence of a radical intermediate. It was concluded from these data that the mechanism of this reaction can be different for different forms of MMO.

There are other data which are in conflict with the oxygen rebound mechanism. It was found [117] that hydroxylation of the stereoisomers of deuterium-substituted camphor by cytochrome $P\text{-}450_{cam}$ is accompanied by partial isomerisation and transfer of deuterium atoms from the *exo*- to the *endo*-position and *vice versa*. This phenomenon is considered as a proof of the radical mechanism. However, the camphor molecule at the active centre of the enzyme is fixed by a hydrogen bond and thus cannot rotate, which follows from the selectivity of this reaction: only 5-*exo*-hydroxycamphor is formed. This means that isomerisation owing to the rotation of the molecule is impossible. It was suggested [117] that a certain radical exists, which can migrate freely and attack the camphor molecule at both the *exo*- and the *endo*-positions of the ring. It must be noted that the presence of this radical in the enzymic reaction is hardly probable.

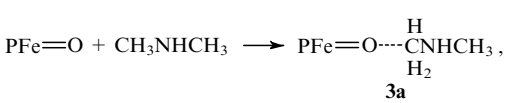
Thus, neither of the two mechanisms mentioned above can explain the body of the experimental data for heme and non-heme monooxygenases altogether. Therefore, the hypothesis of the mechanism of synchronous insertion of the oxygen atom into the C—H bond has been recently supplemented with one important detail, namely: the insertion of oxygen is preceded by the formation of an intermediate complex of the active oxygen with the hydrocarbon molecule. Thus, the oxygen atom plays the crucial role in the complex formation, whereas the metal acts as a ligand at the oxygen atom rather than the centre of complex formation. The complex with active oxygen comprises a strongly distorted substrate structure with pentacoordinated carbon. It is assumed that two hydrogen atoms or one hydrogen and one deuterium atom of this complex can interchange very easily.[118, 119]

If the lifetime of this intermediate is small, *e.g.*, if the O atom is rapidly inserted into the C—H bond, the isomerisation does not take place; if the rate of isomerisation is comparable to that of oxygen insertion, the isomerisation products are formed in appreciable amounts. Thus, the mechanism of this reaction is similar for various enzymes but the ratio of the rate constants for the intermediate isomerisation and the O atom insertion into the C—H bond changes, which results in different selectivities of this reaction. Within the framework of the pentacoordinated carbon mechanism, the unusual stereoselectivity of the hydroxylation of the isotopically substituted camphor in a system with cytochrome $P\text{-}450_{cam}$ has received natural interpretation without any recourse to improbable speculations.[120]

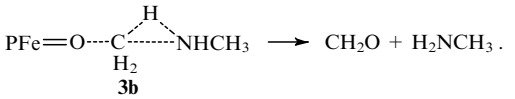
The existence of an intermediate with pentacoordinated carbon has also been postulated in Ref. 116. If this hypothesis is true, the dynamics of hydroxylation of alkanes is qualitatively analogous to the dynamics of hydroxylation of aromatic compounds and epoxidation of alkenes. The same mechanism can also explain the drastic differences in the isotope effects for closely related substrates, such as methane and ethane in the case of MMO. If the hydroxylation occurs in two steps, its rate can be limited by both the first (binding of the O atom to the C—H bond) and the second stage (insertion of the O atom leading to an alcohol) depending on the substrate. Presumably, the maximum isotope effect must be manifested at the second stage. It is believed that it is in the case of methane, the C—H bonds of which are among the strongest, that the rate is determined by the second stage and the isotope effect is a maximum.

A paradoxical situation has been observed earlier for *N*-dealkylation:[36] the isotope effect (8–10) was high for peroxidases the mechanism of which involved an electron transfer, but it was small (1–3) for various forms of cytochrome P-450, chloroperoxidase, and *N*-methylaminomonooxygenase, where the activated O atom directly reacts with the C—H bond.

This paradox can also be overcome by assuming a two-step mechanism involving intermediates with pentacoordinated carbon (**3a** and **3b**), for cytochrome P-450 and its analogues:



3a



3b

The first stage results in the formation of the intermediate **3a**, which is further converted into the intermediate **3b**. This is followed by the second stage, *viz.*, splitting of formaldehyde.

If the rate of this reaction is limited by the first stage, the isotope effect will be small.

The formation of an intermediate complex of the metal-bound oxygen atom with the carbon atom of an unsaturated molecule is beyond doubt for epoxidation of alkenes and hydroxylation of aromatic compounds. At the same time, the absence of saturation in the case of alkane hydroxylation apparently rules out the possibility of formation of an analogous intermediate complex. This new hypothesis is attractive in that it unifies the mechanisms

of atomic oxygen transfer to the C–H bonds of saturated compounds as well as to alkene and aromatic C=C bonds.

III. Models of heme monooxygenases

In addition to monooxygenases, the iron porphyrin complex (FeP) is present in the O_2 carriers (hemoglobin, myoglobin) and enzymes (catalase, peroxidase). One of the reasons for such wide occurrence of the porphyrin system in living nature is its stability. In addition, the porphyrin ring can play the role of an electron donor owing to its ability to generate the radical cation and thus to become an active participant of the catalytic process. Therefore, it was natural to employ metal porphyrins (MP) as catalysts in the simulation of heme monooxygenases. The first model system that involved iron porphyrin (hemin) was proposed in 1972 by Belova (Kulikova) *et al.*[121] Over the past 25 years, studies in this area have continued at a fast pace. This illustrates the fruitfulness of a biomimetic approach in both the analysis of fundamental problems of enzymic catalysis and the design of novel catalysts.[7, 122–124]

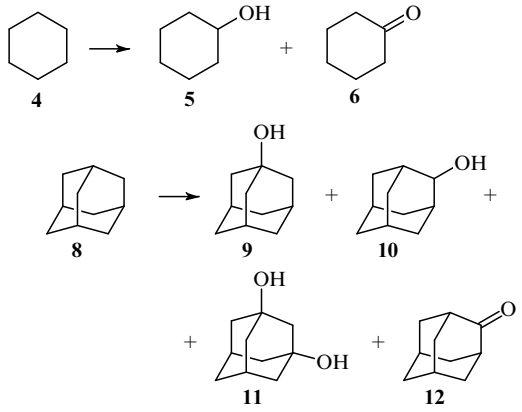
1. Models with active oxygen donors

The simulation of enzymic oxidation with the use of metal porphyrin complexes and active oxygen donors, such as iodosobenzene and its fluorinated analogues, sodium hypochlorite, hydrogen peroxide and alkylhydroperoxides, peroxy acids, potassium persulfate, amine *N*-oxides, and nitrous oxide, has turned out to be very successful.

Iodosobenzene as the active oxygen donor was used for the first time in a biological system.[125] Later, it was employed in a simple system based on iron tetraphenylporphyrinate (FeTPP)[126] responsible for the oxidation of a series of substrates, including alkanes, with a selectivity which is close to that of the enzymic one. A great number of systems initiating alkane oxidation in organic solvents or water–organic solvent mixtures in the presence of phase-transfer catalysts have been described. The most interesting of them are presented in Table 2.

In these systems, cycloalkanes are oxidised under mild conditions to give the corresponding alcohols and ketones (sometimes, alkyl halides) in yields up to 60% with respect to the oxidant consumed and up to 90% with respect to the substrate. The yield of the products, selectivity, and the rate of oxidation depend on the nature of the metalloporphyrin, the axial ligand, the oxygen source, and the substrate. Depending on the system, oxidation of cyclohexane (**4**) gives variable proportions of cyclohexanol (**5**) and cyclohexanone (**6**); the use of NaOCl as the oxidant also yields chlorocyclohexane (**7**). Oxidation of adamantane (**8**) gives adamantan-1-ol (**9**), adamantan-2-ol (**10**), adamantane-1,3-diol (**11**), and adamantanone (**12**) (Scheme 1). The efficiency of the catalyst increases substantially upon introduction of substituents into the *ortho*-position of the phenyl rings of tetraphenylporphyrin. The greatest turnover number (14 600) was observed for halogenated ruthenium tetraphenylporphyrinate,[135] which catalysed alkane oxidation by 2,6-dichloropyridine *N*-oxide (DCPO) at a rate of 800 cycles per min. The virtually complete retention of configuration of the chiral carbon atom and high efficiency open up new opportunities for the application of this system in the stereoselective synthesis of tertiary alcohols.

Scheme 1



Thus, the systems with active oxygen donors are good functional models of heme monooxygenases owing to their ability to oxidise hydrocarbons under mild conditions with the selectivity and at rates which are close to those in enzymic reactions. Another problem of fundamental importance is the nature of the active species that interact with the substrate. As has been mentioned above, a ferryl ($P^{+\bullet}Fe^{IV}=O$) or a perferryl ($PFe^{V}=O$) complex has been usually assumed as an active intermediate in heme monooxygenase systems.

In the case of chemical models, the formation of the ($P^{+\bullet}Fe^{IV}=O$) intermediates was recorded by low-temperature spectroscopy in polar solvents (CH_2Cl_2, CH_3CN), *viz.*, for the interaction of iron tetramesitylporphyrinate (FeTMP) with *m*-chloroperbenzoic acid[103] and for a $PFe^{III}-O_2^{-}-Ac_2O$ system.[136, 137] The only stable $PMn^{V}=O$ complex known so far contains a macrocyclic tetraamide as the ligand.[138]

The intermediates $TF_5PPRu^{II}CO(DCPO)$ and $TF_5PP^{+\bullet}Ru^{II}CO(DCPO)$ were identified in a $Ru^{II}TF_5PPCO$ [ruthenium tetrakis(pentafluorophenyl)porphyrinate]–DCPO system at low temperature.[135] The compound $TF_5PPRu^{III}(DCPO)_2$ was identified in the fast phase of alkane oxidation at 65 °C.

High-valent oxo-complexes of iron porphyrinates were detected in systems with NaOCl, PhIO, and $KHSO_5$; however,

Table 2. Alkane oxidation according to Scheme 1 in the presence of metalloporphyrins with active oxygen donors.

Substrate	Catalyst	Oxidant	Products (ratio)	Yield (%)	Ref.
Cyclohexane (**4**)	MnTFPPCl [a]	NaOCl	**5**+**6**+**7** (1 : 1.3 : 3.8)	24 [b]	127
	MnTDCPPCl [c] + Im [d]	H_2O_2	**5**+**6** (3 : 1)	40 [b]	128
	$Ru^{II}TF_5PP(CO)$	DCPO [i]	**5**+**6** (8 : 1)	95 [g]	135
	MnTDCPPCl + 4-Bu^{t}Py	$KHSO_5$	**5**+**6** (8 : 1)	90 [b]	130
	FeTPPCl [f]	PhIO	**5**+**6** (10 : 1)	26 [g]	131
	$FeTF_5PPCl$ [h]	PhIO	**5**	67 [g]	133
	FeTPPCl	$PhCMe_2OOH$	**5**+**6** (2 : 1)	60 [b]	131
Adamantane (**8**)	$MnCl_{12}TMPCl$ [e]	$KHSO_5$	**9**+**10**+**12** (12 : 5 : 1)	60 [b]	129
	FeTTPCl	PhIO	**9**+**10**+**12** (39 : 5 : 1)	45 [g]	132
	$RuTMP(O)_2$, HCl	DCPO [i]	**9**+**11**+**12**	94	134
	$Ru^{II}TF_5PP(CO)$	DCPO [i]	**9**+**11** (10 : 1)	97 [g]	135

[a] TFPP is tetra(*o*-fluorophenyl)porphyrin; [b] calculated with respect to the substrate; [c] TDCPP is tetrakis(2,6-dichlorophenyl)porphyrin; [d] Im is imidazole; [e] TMP is tetramesitylporphyrin; [f] TPP is tetraphenylporphyrin; [g] calculated with respect to the oxidant consumed; [h] TF_5PP is tetrakis(pentafluorophenyl)porphyrin; [i] DCPO is 2,6-dichloropyridine *N*-oxide.

their structures differed from that of $P^{+\bullet}Fe^{IV}=O$ in all cases. According to their absorption spectra, these compounds were characterised either as $PFe^{IV}=O$[103,139–141] or ($PFe^{IV}-O)L$,[142–145] although the presence of the ligand L in the coordination sphere of the metal has not been established. Similarly, for manganese porphyrinates–NaOCl systems, the length of the Mn–O bond in the active species determined by the EXAFS method is more consistent with an Mn–O–Cl structure.[145] As the $PMn^{IV}=O$ and $PFe^{IV}=O$ complexes can oxidise alkenes but are inactive towards alkanes,[146,147] their monooxygenase function is usually associated with their ability to generate more reactive intermediates (formally, pentavalent metal oxo-complexes) at room temperature. The latter cannot be detected by physicochemical methods because of their extremely short lifetime under these conditions.

Other active oxygen donors are less suitable for the preparation of high-valent oxo-complexes of metalloporphyrins: anhydrous hydrogen peroxide destroys iron complexes even at −30 °C;[148] *tert*-butyl hydroperoxide generates cationic isoporphyrins with the ROO-group linked at one of the *meso*-positions.[149]

Systems with H_2O_2 and ROOH can generate several reactive species, such as hydroxyl or alkoxyl radicals and metal oxo-complexes. Homolytic cleavage of the O–O bond in hydrogen peroxide or alkyl hydroperoxide coordinated with metalloporphyrin yields $PM^{IV}=O$ and free radicals ($HO^{\bullet}$ or $RO^{\bullet}$). Heterolytic cleavage generates $P^{+\bullet}Fe^{IV}=O$ and water (or the corresponding alcohol).

In systems based on cumyl and *tert*-butyl hydroperoxides with iron or manganese porphyrins, the kinetic characteristics and the selectivity of the reaction are independent of the nature of the metalloporphyrin; hence it was concluded[131,150,151] that free radicals $RO^{\bullet}$ and $ROO^{\bullet}$ are reactive species in this process.

Introduction of imidazole (Im) into a manganese tetrakis(dichlorophenyl)porphyrinate (TDCPPMn)–H_2O_2 system changes the kinetic features and the composition of the oxidation products. The high activity of this system was explained[152] by the fact that imidazole used as a nucleophilic axial ligand favoured heterolysis of the O–O bond with the formation of $PMn^{V}O$ complexes and facilitated this cleavage as an electrophilic acid catalyst. The effect of axial ligands on the catalytic function of metalloporphyrins (up to complete suppression of epoxide formation) has also been observed in biomimetic systems of alkene oxidation by monopersulfate.[153,154] Cleavage of the O–O and one of the O–H bonds in hydrogen peroxide coordinated with metalloporphyrin is the rate-determining step in the reaction of metalloporphyrins with H_2O_2.[147,155] Traylor *et al.*[156] have suggested that the O–O bond in a hydroperoxide coordinated with metalloporphyrin (as in the case of protic solvents) is always cleaved heterolytically to yield $PFe^{V}=O$, which further reacts with alkenes or hydrogen peroxide. The interaction with peroxides (H_2O_2, ROOH) gives rise to the radical intermediates ($HO^{\bullet}$, $HO_2^{\bullet}$, $RO^{\bullet}$, $RO_2^{\bullet}$). By increasing the electronegativity of iron porphyrinate (by introducing substituents into the porphyrin ring), Traylor *et al.*[156] succeeded in performing the reaction of $PFe^{V}=O$ preferentially with alkenes and thus increased the epoxide yield up to 60%–100% with respect to H_2O_2.

2. Models with molecular oxygen

The use of bound oxygen as the oxidant allows one, as a rule, to carry out the reactions with high selectivity and simplifies the analysis of reactive intermediates. However, the use of halogen-containing oxidants leads to the contamination of the reaction products with organohalogen compounds and other impurities. In this context, molecular oxygen is the oxidant of choice both from the ecological and the economic points of view.

It was found that Mn, Fe, and Cr tetramethylporphyrinates with azide as the axial ligand catalyse isobutane autooxidation to give *tert*-butyl alcohol at 80 °C with a selectivity of 88%–92%.[157–159] The activity of the catalyst increases abruptly with a substitution of the hydrogen atoms in the porphyrin ring by halogen atoms.[160,161] When iron *meso*-tetrakis(penta-fluorophenyl)-β-octabromoporphyrinate ($FeTPPF_{20}$-β-Br_8) was used as the catalyst, isobutane oxidation proceeded with a selectivity of 92% at room temperature; the catalyst turnover number exceeded 13 000 over 70 h and the catalytic activity was retained for 74 h. However, such efficiency was observed only for the alkanes with tertiary C–H bonds. The system is far less efficient for secondary C–H bonds and is completely inactive with respect to primary C–H bonds.

High selectivity and the fact that no co-catalysts are needed have raised great interest in these systems. Analysis of the absorption spectra recorded at room temperature has revealed that $Fe^{II}TF_5PPCl$ and a Fe-μ-oxodimer $(FeTF_5PP)_2O$, which can be isolated from the solution, are formed in a $FeTF_5PPCl-O_2-C_6H_6-iso\text{-}C_4H_{10}$ system.[160,161] Non-halogenated FeTPP produce inactive μ-oxo complexes that do not catalyse oxidation of saturated hydrocarbons. Ellis and Lyons[161] have postulated the generation of $PFe^{IV}=O$ as the reactive species in this system. However, further studies have demonstrated that the reaction of hydroperoxides with Fe^{II} yields free radicals, which further initiate the chain process.[162]

3. Models with molecular oxygen and a reducing agent

Simulation of the key reaction of monooxygenase catalysis, *viz.*, the activation of molecular oxygen by a metal complex in the presence of an electron donor (reducing agent), is undoubtedly of theoretical and practical interest.

Superoxo- and peroxo-ferryl complexes synthesised from O_2 and iron porphyrinate (PFe) in the presence of a reducing agent are relatively stable compounds, which are inert with respect to alkanes. It was only the introduction of an additional component (acetic anhydride) into the system that has resulted in the first complete chemical model of cytochrome P-450 on the basis of PFe that was active towards saturated hydrocarbons.[163] The role of the effector in this system was played by acetic acid that was present in admixture with acetic anhydride purified using conventional procedures.[164,165]

Examples of these model systems are given in Table 3. Hydrogen in combination with colloidal platinum, sodium ascorbate, dihydropyridine, zinc (powder or amalgam), and electrolysis were used for reduction and a mineral or a carboxylic acid or water as the proton donor.[163–168]

Alkane oxidation in the presence of O_2 and an electron (and proton) source occurs at normal temperature and pressure. The selectivity and the rate of the reaction depend on the nature of the metalloporphyrin as well as on the electron and proton donors. The product yield with respect to the reducing agent is usually low. The best yield (37%) was obtained in the oxidation of hydrocarbons having weak C–H bonds (indane and tetralin) with Zn as the reducing agent.[168] In non-enzymic systems, it is difficult to obtain high yields of the reaction products with respect to the reducing agent, since the reactive oxidant formed in this process reacts with the substrate and the reducing agent with equal ease. Model systems based on molecular oxygen and the reducing agent are usually less selective than the systems in which active oxygen donors play the role of the oxidants.

Nevertheless, the obvious advantage of such systems is a high oxidation rate, which sometimes exceeds that of enzymic reactions by more than one order of magnitude.

As in the catalytic cycle of cytochrome P-450, the generation of reactive intermediates in biomimetic systems based on molecular oxygen and the electron donor occurs in several consecutive steps. The first step, which is usually rate-limiting, is the reduction of metalloporphyrin. The reduced metalloporphyrins add oxygen to yield a dioxygen complex with a rate constant of the order 10^7 M^{-1} s^{-1} (see Ref. 169). This reaction is reversible but the $TpivPPFe^{II}(O_2)(Im)$ complex could be isolated if sterically hindered iron tetrakis(*o*-pivaloylaminophenyl)porphyrinate

Table 3. Cycloalkane oxidation in systems with O_2 and a reducing agent.

Substrate	Catalyst	System	Solvent	Products (ratio)	Number of catalytic cycles /min^{-1}	Ref.
Adamantane (**8**)	MnTPPCl[a] + MeIm[b]	H_2/Pt – HCl – $(PhCO)_2O$	MePh – EtOH	**9** + **10** + **12** (15 : 1.7 : 1)	0.004	166
		Zn dust – AcOH	MeCN – CH_2Cl_2	**9** + **10** + **12** (20 : 2 : 1)	0.5	168
Cyclooctane	MnTPPCl + MeIm	Zn dust – AcOH	MeCN – CH_2Cl_2	cyclooctanol + + cyclooctanone (1.8 : 1)	0.5	168
Cyclohexane (**4**)	MnTPPCl	sodium ascorbate	C_6H_6	**5** + **6** (1 : 7.5)	0.006	167
	hemin[c] + NaOH	thiosalicylic acid	$(Me)_2CO – H_2O$	**5** + **6** (1.8 : 1)	0.4	121
	FeTpivPPCl[d]	Zn dust – AcOH	MeCN	**5** + **6** (1.7 : 1)	2	164
	FeTpivPPCl	Zn dust – AcOH – MV[e]	MeCN	**5** + **6** (3.3 : 1)	50	164
	FeTPPCl	Zn dust – AcOH – MV	MeCN	**5** + **6** (2 : 1)	340	164
	FeTPPCl	Zn dust – PhCOOH – MV	MeCN	**5** + **6** (1.5 : 1)	340	164

[a] TPP is tetraphenylporphyrin; [b] MeIm is *N*-methylimidazole; [c] hemin is a natural iron-containing protoporphyrin IX; [d] TpivPP is tetrakis(*o*-pivaloylaminophenyl)porphyrin; [e] MV is methyl viologen.

(FeTpivPP) and *N*-methylimidazole (MeIm) or 1-tritylimidazole (TrIm) were used.[170, 171]

In the presence of a reducing agent, the addition of oxygen to the reduced metalloporphyrin is followed by the reduction of the oxygen complex. The rate constant for the reduction of $TpivPPFe^{II}(O_2)(TrIm)$ by methylviologen (MV) radical cation is equal to $2.2\pm0.4\times10^9$ M^{-1} s^{-1} (Ref. 172).

Protonation of the oxygen atom of the peroxo group results in the homolytic or heterolytic cleavage of the O – O bond depending on the pH of the medium and the polarity of the solvent (similarly to the systems based on hydrogen peroxide).[173] However, no direct correlation between solvent polarity and the homolysis or heterolysis of the O – O bond in metalloporphyrin intermediates has been found.

The kinetics and the mechanism of generation of reactive species that interact with alkanes have been extensively studied for metalloporphyrin – O_2 – carboxylic acid – Zn systems.[165, 174] The configuration of the chiral carbon atom of *cis*- or *trans*-1,2-dimethylcyclohexane is not retained in the catalysis by iron porphyrins. The competitive oxidation of C_6H_{12} and C_6D_{12} in this system has a relatively low KIE (2 – 3). These data permit one to postulate, with an equal probability, both a radical and a molecular mechanism. Analysis of substrate selectivity in the competitive oxidation of cyclopentane and cyclohexane (the so-called 5/6 parameter) was more informative. The dependence of this parameter on the nature of substituents in the phenyl rings of iron porphyrinate, and its magnitude (0.38 – 0.50), which is characteristic of an electrophilic attack by a positively charged reagent,[175] suggest that the ferryl ($P^{+\bullet}Fe^{IV}=O$) or perferryl ($PFe^{V}=O$) complexes are the reactive species.

The ratio of the rate constants for the attack at the C – H bond of cyclopentane and cyclohexane (the 5/6 parameter) is being employed more and more often for the study of mechanisms of various reactions of cycloalkanes and alkanes. It is reasoned[175] that this parameter is a measure of the partial charge on the carbon that is attacked in the transition state. The 5/6 ratio is close to unity and does not depend on the reagent charge in reactions where H is detached by a radical or a radical ion. In the case of an electrophilic attack by a positively charged reagent, this parameter is equal to, or less than, 0.5 depending on the reagent charge.[175]

In the catalysis by MnTPPCl in an O_2 – carboxylic acid – Zn system, the value of the parameter 5/6 (0.7) is close to that observed in the absence of the metalloporphyrin. However, partial retention of configuration (25%) in the oxidation of *cis*- or *trans*-dimethylcyclohexanes shows that the reaction catalysed by MnTPPCl is not, at least partly, a free-radical process. Oxidation of alkenes and the regioselectivity of n-heptane oxidation in MnTPPCl – AcOH – Zn – MeIm and PhIO – MnTPPCl – MeIm systems follow the same regularities.[168]

The experimental results altogether lead to a conclusion about a single mechanism of the generation of reactive species in the systems based on iron and manganese porphyrins with molecular oxygen and proton and electron donors. The candidates for the reactive intermediates in these systems are high-valent oxo-complexes of metalloporphyrins formed by reductive activation of molecular oxygen in the presence of a proton donor *via* a catalytic cycle that is similar to that of cytochrome P-450. The reactive species in these systems, as well as in the systems with different active oxygen donors may differ from each other depending on the starting reagents. This follows from the differences in the stereoselectivity and KIE in different systems that generate reactive metalloporphyrin complexes.[122]

4. Models with molecular oxygen and an aldehyde

It was found[176] that cyclohexane can be efficiently oxidised to give cyclohexanol and cyclohexanone by molecular oxygen in the presence of halogenated metalloporphyrins and propionaldehyde at 30 °C. The relative yields of the oxidation products depends on the nature of the central ion and the porphyrin ligand of the catalyst. The maximum turnover number for the catalyst (24) and the maximum yield with respect to the substrate (5%) were obtained for manganese *meso*-tetrakis(2,6-dichlorophenyl)-β-octachloroporphyrinate (MnTDCPP-β-Cl_8). The ketone/alcohol ratio varied from 0.8 for FeTDCPP to 4.3 for MnTDCPP-β-Cl_8 depending on the conditions. After completion of the reaction, an appreciable amount of the surviving catalyst was found only in the case of halogenated manganese porphyrinate. This system involves two oxidants, *viz*., molecular oxygen and a peroxy acid,[176–178] which is formed according to the scheme:

$$PM^n + RCHO \longrightarrow PM^{n-1} + RCO^{\bullet} + H^+,$$

$$RCO^{\bullet} + O_2 \xrightarrow{RCHO} RCOOOH.$$

The aldehyde acts simultaneously as the reducing agent for the metalloporphyrin and the source of an effective oxidant. The peroxy acid reacts with the metalloporphyrin either to form high-valent oxo-complexes [pathway (*a*)] or to decompose *via* a radical mechanism [pathway (*b*)].

$$PM^n + RCOOOH \xrightarrow{a} PM^{n+2}{=}O + RCOOH$$

$$PM^n + RCOOOH \xrightarrow{b} PM^{n+1}{=}O + RCOO^{\bullet} + OH^-$$

$$RCOO^{\bullet} \longrightarrow R^{\bullet} + CO_2.$$

Thus, according to this scheme,[176–178] both high-valent oxo-complexes of metalloporphyrin and free radicals are the reactive species in this system. In addition, a possibility of direct inter-

action of a hydrocarbon with metalloporphyrins having a high oxidation potential (*e.g.*, PCo [111]) can also be considered:

$$PM^n + RH \longrightarrow [PM^{n-1} + RH^{+\bullet}] \longrightarrow PM^{n-1} + R^{\bullet} + H^+.$$

It is noteworthy that this oxidation mechanism is mostly hypothetical and demands further investigation.

5. Photocatalytic models with O_2

Irradiation of cycloalkanes with light of wavelength ~350–450 nm initiates their oxidation by molecular oxygen (20 °C, 200 Torr O_2) in the presence of iron complexes with halogenated tetraphenylporphyrins. In this system, cycloalkanes are oxidised to the corresponding alcohols and ketones. The turnover number of the catalyst does not exceed 230 due to its destruction.

The selectivity of cyclohexene oxidation is distinguished from enzymic processes: epoxides are not produced, while the corresponding alcohol and ketone are formed in high yields (5000 and 4500 reaction cycles for cyclohexenol and cyclohexenone, respectively). Based on these data, a free-radical oxidation mechanism has been proposed for this system. Presumably, hydroxyl radicals generated in the photoactivation of $PFe^{III}-OH$ and peroxy radicals $ROO^{\bullet}$ formed in the reaction of alkyl radicals with oxygen are the reactive species.[179]

Thus, in models of heme monooxygenases with various oxidants, alkanes can react under mild conditions due to their interaction with active radicals or high-valent metal complexes. The KIE and the stereoselectivity of all biomimetic systems generating high-valent oxo-complexes of metalloporphyrins vary considerably depending on the nature of the metal and the substituents in the phenyl rings of the metalloporphyrins, the nature of the active oxygen donor, and even the properties of the reaction medium.[122]

There are two viewpoints as to whether the observed variations reflect differences in the structures of reactive intermediates. Some authors [141, 148] believe that it is the $(P^{+\bullet}M^{IV}O)$ complex formed in all these systems (*e.g.*, by disproportionation of two ferryl complexes) that is the species that gets alkanes involved in the oxidation. According to Shteinman [180] and Hill [181] the reactive complexes that react with alkanes may vary depending on the structure of the original reagents and the reaction conditions. The differences in the stereoselectivity and the magnitude of KIE in systems with different oxygen donors point to differences in the structures of reactive intermediates. Analysis of the experimental data altogether for various types of systems provides a simple explanation for the effects of different factors (polarity, acidity or basicity, the presence of axial ligands in metalloporphyrins, *etc.*) on the structure of the reactive intermediates and, correspondingly, their reactivity with respect to different substrates.

It should be noted that the reactive complexes, $P^{+\bullet}M^{IV}O$, bear a positive charge. Depending on the medium and the nature of the ion, different species can exist in the solution, such as free ions $(P^{+\bullet}M^{IV}O)$ (in polar media) and ion pairs $(P^{+\bullet}M^{IV}O)^+L^-$ (in less polar and non-polar media). Naturally, their activity towards alkanes will also be different. Nucleophilic and electrophilic properties of the complex and the environment are important for the formation of $(P^{+\bullet}M^{IV}O)^+$, which is more reactive than $PM^{III}OL$. Axial ligands, such as imidazole, will play the role of nucleophiles, while acids will favour the heterolytic cleavage of the O–L bond.

Thus, the model systems based on metalloporphyrins catalyse the oxidation of hydrocarbons in organic solvents; the parameters of this process (temperature, reaction rate) are in many cases similar to those observed in enzymic reactions. In the absence of the stabilising effect of the protein, metalloporphyrins undergo decomposition under the oxidation conditions, which results in the loss of the catalytic activity during the reaction. Analysis of the experimental data accumulated to date demonstrates that halogenation of the porphyrin macrocycles increases the stability and the catalytic activity of metalloporphyrin complexes. Halogenated metalloporphyrins containing halogen atoms both in the phenyl and the pyrrole rings are superior in this respect. Halogenation enhances the oxidation potential both of the metal centre and of the porphyrin ligand. This, in turn, increases the stability of the complex under the oxidation conditions and the electrophilicity of the metal centre, and, correspondingly, the catalytic activity of the metalloporphyrin complex.

One of the ways of increasing the selectivity of biomimetic systems is the use of porphyrin ligands with bulky groups that create steric hindrances around the active centre. The molecular design of porphyrins is becoming more perfect in this aspect. Recently, a method for the synthesis of a new family of functional models of hemes (the so-called *double picket fence*) with chiral surfaces on both sides of the plane of the porphyrin rings has been proposed.[182] The use of spatially organised porphyrin complexes strongly increases the proportion of primary alcohols and aldehydes in the products of oxidation of linear alkanes, thus simulating ω-hydroxylation, which is a characteristic feature of enzymic catalysis.[29, 183–185]

Another way of increasing the selectivity and the stability of catalysts is the simulation of the microenvironment of the active centre of enzymes through the construction of organised molecular systems. This term refers to multicomponent catalytic systems with an optimum spatial arrangement and energetic and orbital compatibility between the components of the catalytic system and the substrates. Some general principles for the design of organised molecular species, *viz.*, the models of cytochrome P-450, have been formulated in order to achieve optimum rates and selectivity of the monooxygenase reaction.[186] Methods of molecular design are widely employed for enhancement of various characteristics of the chemical models.

6. Metalloporphyrins in microheterogeneous environments and immobilised on supports

a. Micelles

The selectivity of cyclohexane oxidation in an iron tetrakis-(*p*-hexadecyloxyphenyl)porphyrinate (FeTH–DOPP)–PhIO microheterogeneous system is higher than that of the homogeneous analogue (cyclohexanol becomes the only product) but the rate of the reaction decreases.[142]

Different reactive species have different regioselectivities. To estimate it, the parameter 1 °/2 °/3 °, *i.e.*, the ratio of the products of the attack at the primary, secondary, and tertiary C–H bonds with allowance made for the number of bonds of each type, has been proposed.[187] In the case of metal complexes, steric factors can play an important role. Therefore, metal complexes can attack

Table 4. The regioselectivity of n-hexane oxidation by metalloporphyrin chemical enzymic systems.

Catalyst	Oxidant	Medium	Selectivity index [a]	Ref.
FeTHDOPPCl [b]	PhIO	C_6H_6	0.003	142
	PhIO	H_2O–Triton X-100	0.021	
	PhIO	H_2O–DMPCh [c]	0.050	
	PhIO	H_2O–CD [d]	0.211	
	PhIO	H_2O–MeCD [e]	0.234	
MnTPPOAc	PhIO	C_6H_6	0.027	150
MnTTMPPOAc [f]	PhIO	C_6H_6	0.041	
MnTTPPPOAc [g]	PhIO	C_6H_6	0.310	
Cytochrome P-450 (CYP2B4)	O_2	H_2O	0.160	188

[a] The regioselectivity index is equal to the ratio of the yields of oxidation products of primary and secondary C–H bonds taking into account the ratio of these bonds in the hexane molecule; [b] THDOPP is tetrakis(n-hexadecyloxyphenyl)porphyrin; [c] DMPCh is dimyristoyl phosphocholine; [d] CD is cyclodextrin; [e] MeCD is methylcyclodextrin; [f] TTMPP, is tetrakis(2,4,6-trimethoxyphenyl)porphyrin; [g] TTPPP is tetrakis(2,4,6-triphenylphenyl)porphyrin.

a methyl group or an adjacent methylene group more easily than the less accessible methine group or a CH_2 group inside a polymethylene chain.

Table 4 presents data on regioselectivity of hexane oxidation by cyclodextrin (CD) microheterogeneous model systems and (for comparison) enzymes. The regioselectivity with respect to the terminal group increases with an increase in the degree of ordering of the system by nearly two orders of magnitude in comparison with the homogeneous analogue. It is seen that the selectivity of certain microheterogeneous systems is close to that of the most regioselective isoform of cytochrome P-450$_{LM2}$ (CYP2B4). Apparently, the molecules of hydrophobic PFe and alkane in these organised molecular systems are specifically arranged relative to one another in such a way that the reaction at the terminal CH_3 group becomes preferential.

b. Metalloporphyrins in a phospholipid bilayer

Groves and Neumann [189] have developed a system with an optimum spatial arrangement based on membrane-bound Mn tetrakis[*o*-(cholenylamido)phenyl]porphyrin, Mn^{III}(ChP)Cl, which simulates the hydrophobic microenvironment of the active centres of enzymes. Being incorporated into phospholipid vesicles, the catalyst occupies the central position in the lipid bilayer in such a way that the plane of the porphyrin ring becomes parallel to the water surface. Both molecular oxygen in the presence of a reducing agent and sodium periodate can be used as alkane oxidants.

Selective hydroxylation of the C(25) carbon atom of cholesterol, which does not involve the more reactive C(5) = C(6) double bond of the molecule, is the most striking result of the application of molecular design principles to this particular system. Groves and Neumann [189] attribute this effect to the proximity of the tertiary carbon atom C(25) to the metal in the centre of the complex. The selectivity of the process depends on the oxidant. The reaction rate is very low, but the catalyst retains its stability over a long period of time.

c. Metalloporphyrins immobilised on supports

The number of a catalyst's reaction cycles is usually restricted due to the drop of its activity in the course of oxidation. This problem has been solved in Groves' molecular organised systems, but the system itself is extremely complex. Another approach to the simulation of the catalyst's microenvironment is its immobilisation on a solid support, *i.e.*, the formation of heterogeneous oxygenase systems. Examples of such systems are given in Table 5.

Immobilisation of a metalloporphyrin on porous glass or polyvinylpyrrolidone (PVP) markedly increases the selectivity of alcohol formation. Immobilisation of an iron – porphyrin complex on zeolites [188] and especially on graphite, strongly increases the steric effects, which are observed in hexane oxidation in model systems with active oxygen species.[192] The effect of the matrix is not confined to the increase in the steric hindrances around the active oxidant. It can also be accompanied by a sieve effect, which is well-known for zeolites and accounts for the differences in the substrate specificity.

Heterogeneous systems of alkane photooxidation by oxygen in the presence of iron and manganese porphyrinates immobilised on TiO_2-modified montmorillonite have also been developed.[193]

As expected, the heterogenisation of metalloporphyrin catalysts is accompanied by a decrease in the rate of hydrocarbon oxidation. At the same time, heterogeneous systems have significant advantages over their homogeneous analogues. Immobilisation of metalloporphyrins on various supports not only enhances the selectivity of the reactions catalysed by them but also increases their stability in the majority of cases. For example, immobilisation of iron porphyrinate on porous glass [164] increased the number of reaction cycles nearly 200-fold.

It is generally accepted that the reactive species in systems with metalloporphyrins immobilised on supports are similar to those in solution. Apparently, in most cases this conclusion reflects the real situation; however, it should be kept in mind that binding of the catalyst with the support can change its coordination properties and the ability to produce reactive intermediates.

7. The mechanism of alkane oxidation in systems based on metalloporphyrins

The transfer of the oxygen atom by porphyrin complexes to C – H bonds in natural and model systems is a matter for debate. Various types of molecular mechanisms and a crypto-radical oxygen rebound mechanism have been considered in the context of enzymic hydroxylation. In model systems where the yield of radicals into bulk solution is not excluded, an ordinary radical process can also occur *via* the intermediate formation of a hydro-

Table 5. Alkane oxidation catalysed by metalloporphyrins immobilised on supports.

Substrate	Oxidant	Catalyst	Medium or support	Product ratio alcohol : ketone	Number of catalytic cycles	Ref.
n-Octane	PhIO	$MnCl_{12}$TSMPPOAc [a]	solution	1.8	42	190
			PVP [b]	2.4	24	
			[PVPMe][TsO] [c]	11.7	76	
Adamantane (**8**)	PhIO	$MnCl_{12}$TSMPPOAc	PVP	21	238	190
			[PVPMe][TsO]	53	322	
			amberlite IRA-900	16	50	
	PhIO	Mn(T4MPyP)Cl [d]	solution	14	1.5	191
			SiO_2	37	7.6	
			clay (5%)	28	11.8	
Cyclooctane	PhIO	Mn(T4MPyP)Cl	solution	0.8	2.2	191
			SiO_2	5.2	8.1	
			clay (5%)	8.8	11.8	
Cyclohexane (**4**)	PhIO	Mn(T4MPyP)Cl	solution	2.0	1.2	191
			SiO_2	6.5	6.0	
			clay (5%)	8.8	7.8	
	O_2, Zn – AcOH – MV [e]	FeTHDOPPCl [f]	solution	2	10	164
			porous glass	5	1800	
	O_2, aldehyde	CoTMeP [g]	SiO_2	0.53	14.7	176

[a] Cl_{12}TSMPP is tetrakis(3-chloro-5-sulfo-2,4,6-trimethylphenyl)-β-octachloroporphyrin; [b] PVP is polyvinylpyrrolidone; [c] [PVPMe][TsO] is polyvinylmethylpyrrolidone tosylate; [d] T4MPyP is tetrakis(4-*N*-methylpyridyno)porphyrin; [e] MV is methyl viologen; [f] THDOPP is tetrakis(n-hexadecyloxyphenyl)porphyrin; [g] TMeP is tetramethylporphyrin.

peroxide; the final products of its conversions are an alcohol and a ketone.

High values of KIE and isomerisation of the molecules subject to oxidation are usually regarded as proofs of the oxygen rebound mechanism both in natural and model systems.[132] However, despite the seemingly convincing character of these arguments, a careful scrutiny of the experimental data has demonstrated a discrepancy in this concept for biomimetic systems.

Isomerisation observed in the hydroxylation of aromatic compounds (the NIH shift) and in the epoxidation of alkenes (simultaneous formation of aldehydes and epoxides) in chemical and enzymic systems points to the existence of reactive intermediate complexes (rather than free radicals). The effect of radical traps on alkane oxidation may also indicate both the generation of free radicals in parallel and the formation of an intermediate complex in which the alkyl group may acquire sufficient mobility for its reaction with the traps. In addition, the thermodynamic feasibility of radical formation, especially in the case of a strong C–H bond, raises certain doubt. Calculations have shown[194] that if a purely radical mechanism is realised in the first stage, high rates of the reaction of high-valent metal complexes with alkanes can be reached only in the case of weaker tertiary C–H bonds.

The value of the KIE is widely used in the interpretation of mechanisms as the criterion of hydrogen transfer in the form of H^+, H^-, or $H^\bullet$ in the limiting step as well as in the analysis of the transition state structure.[100,195] Small values of KIE ($\sim 1-2$) have been determined for oxidation reactions involving a very reactive (and therefore very non-selective) $^\bullet$OH radical. Higher values of KIE point to the involvement of a more selective intermediate (*e.g.*, metallooxene) as the active oxidant.

For many metalloporphyrins as well as for cytochrome P-450, hydroxylation of C–H bonds in the presence of atomic oxygen donors is characterised by a high isotope effect ($k_H/k_D > 9$). As in the case of natural monooxygenases, for their chemical models[138,186,196] it was assumed that the formation of the ($POH + R^\bullet$) pair results from the transfer of the hydrogen atom from the substrate to the reactive intermediate, while the differences in the KIE values are accounted for by a different degree of linearity of transition states. High value of k_H/k_D must correspond to a symmetrical linear transition state for the transfer of the H atom. At the same time, theoretical calculations show that the transition state becomes practically linear after detachment of the hydrogen atom from the methane molecule by H and O atoms or by the $^\bullet$OH radical, but the experimental values of the KIE are small in this case. Moreover, it was established that in some model systems based on metalloporphyrins and active oxygen donors, the experimental values of the KIE substantially exceed the maximum values calculated from the difference in zero point energy levels of the C–H and C–D bonds ($k_H/k_D \sim 7-8$ at 20 °C).[195] The high value of the KIE (> 9 at 25 °C) may indicate a contribution to the mechanism of proton tunnelling (rather than that of the deuteron, which is heavier than the proton and less prone to tunnelling).[100] Obviously, the tunnelling conditions develop gradually but not spasmodically. It is natural to speculate that in all the reactions that occur by this mechanism, the tunnelling effect is present to a greater or a lesser degree, this should be observed for high and narrow barriers.

Hence, a question arises as to why the KIE is so different in the hydroxylation reactions occurring *via* a similar mechanism. It should be noted that in reactions with a high KIE ($k_H/k_D > 6$) its increase is accompanied by an increase in stereoselectivity.[174] At the same time, for systems with a relatively low KIE (about 2), the stereoselectivity varies over a very wide range: from 0% for the FeTPPCl–O_2–Zn–AcOH system to 90% for the MnTDCPPCl–$KHSO_5$ system.

The effect of the medium polarity on the KIE can be accounted for by the lowering of the potential barrier with an increase in ionisation and by the tunnelling effect. In more polar media, the proton is somewhat 'heavier', being more strongly solvated by the solvent molecule, and the ratios of the masses of the solvated protons and deuterons are smaller than those of the free ones.

Apparently, direct interaction of a high-valent metalloporphyrin complex with the C–H bond of the substrate, which is accompanied by the insertion of the O atom into the C–H bond *via* an intermediate complex with a pentacoordinated carbon is the most probable mechanism for the systems that simulate biological hydroxylation. That the direct insertion is possible follows from the complete retention of the original configuration of the tetrahedral carbon atom observed in some cases, *e.g.*, in the hydroxylation of 1,2-dimethylcyclohexane by an iron–porphyrin complex in a biphasic water–benzene system in the presence of NaOCl.[142–145]

Isomerisation, which is sometimes observed in the oxidation and which is usually considered as evidence in favour of the intermediate formation of radicals, is often in contradiction with the radical mechanism on closer inspection. Thus the hydroxylation of *all–exo*-2,3,5,6-tetradeuterionorbornane using the *meso*-tetrakis(2,6-dichlorophenyl)-β-octabromoporphyrin complex (Br_8TDCPPFe) with pentafluoroiodosobenzene[197] was accompanied by a partial transfer of deuterium to the *endo*-position. This was interpreted by intermediate formation of a norbornyl radical; however, analysis of the distribution patterns of the hydroxylation products has led to a conclusion that the probabilities of the rotation of the radicals generated by hydrogen detachment from the *exo*- and *endo*-positions of the ring differ drastically (by more than one order of magnitude). If it is assumed that the isomerisation occurred within the intermediate complex in which the carbon atom coordinates the oxygen atom linked with the Fe atom, then it follows from the same data that the rotation leading to the isomerisation is equally probable when the oxygen is added at both the *exo*- and the *endo*-positions. In our opinion, the second mechanism is preferable, especially if one takes into consideration the results obtained for the natural cytochrome P-450.

Hydroxylation of *R*- and *S*-enantiomers of monodeuterioethylbenzene with iodosobenzene in the presence of a chiral iron–porphyrin complex, which yields all possible isomers of 1-phenylethanol and its deuterioisotopomers, has been studied.[198] It has been found that the main product is formed stereoselectively in both cases and is virtually pure monodeuteriophenylethanol, whereas the deuterium content in the minor product is only 50%. In order to explain these results within the framework of the oxygen rebound mechanism, Groves and Viski[198] had to assume that the intermediate radical formed rotates with a marked probability only in the detachment of H from the pro-*S*-position but does not rotate at all when the detachment occurs from the pro-*R*-position. If one admits the mechanism of oxygen insertion with intermediate formation of a pentacoordinated carbon, the observed results can be easily explained by the isotope effect observed in the insertion of the O atom into the C–H and C–D bonds. If the isotope effect is great, the rate of its insertion into the C–H bond can be much higher than the isomerisation rate, whereas for the C–D bond the rates of the insertion and isomerisation are comparable.

Quantitative estimation of the relative rates of insertion and isomerisation for this reaction[120] gave a correlation between the rate constants for addition, insertion, and isomerisation. The parameters obtained permit comparison of the experimental and the calculated relative yields of the hydroxylation products (see Table 6). Complete agreement of the experimental and calculated ratios of the reaction products provides evidence for the proposed mechanism. Indeed, the ratio of the reaction products, *e.g.*, for (*S*)-deuterioethylbenzene, can be predicted from the parameters calculated for the *R*-isomer without any additional postulates, and the calculated and the experimental values coincide.

Table 6. The relative yields of the isomers and the deuterioisotopomer of 1-phenylethanol during the oxidation of (*R*)- and (*S*)-(1-deuterioethyl)benzene.[120]

Substrate	Isomer [a]	Relative yield (%)	
		experimental [198]	calculated [120]
(*R*)-(1-Deuterioethyl)benzene	R_H	23	22.9
	R_D	19	19.1
	S_H	1	1.1
	S_D	57	56.9
(*S*)-(1-Deuterioethyl)benzene	R_H	2	1.7
	R_D	87	87.3
	S_H	6	6.0
	S_D	5	5.0

[a] The H and D indices are related to the products of oxidation of the C–H and C–D bonds, respectively.

It can be noted that theoretical calculation [199] shows that triprotonated methane, CH_7^{3+} (*i.e.*, heptacoordinated carbon) can, in principle, exist; other authors [200, 201] have succeeded in obtaining complexes of penta- and hexa-coordinated carbons as stable crystalline salts, $\{[(C_6H_5)_3PAu]_5C\}^+$ and $\{[(C_6H_5)_3PAu]_6C\}^{2+}$.

Thus, the non-radical mechanism of oxidation, which involves the attachment of the oxygen atom at the C–H bond and its subsequent insertion into this bond, provides quantitative characteristics for the rationale of the unusual distribution of the reaction products and the isotope effects of hydroxylation of isotopically modified molecules in enzymic and biomimetic systems in which H and D atoms interchange in the course of hydroxylation. Analysis of the rates of the separate stages of this reaction calculated for the new mechanism has revealed interesting and unusual dynamics of the hydroxylation process. The kinetic parameters give a noncontradictory pattern of the reaction dynamics without any recourse to improbable statements, which distinguishes it favourably from the oxygen rebound mechanism employed so far.

Thus, the systems based on metalloporphyrins discussed above not only represent functional models of heme monooxygenases. Many of them simulate the natural process both as regards the reactive species and the oxidation mechanism. Recent data demonstrate significant advances in the design of novel systems based on metalloporphyrins and shed additional light on the mechanisms of reactions occurring in them, although the proposed oxidation schemes are often hypothetical. Further investigations are necessary in order to create more efficient systems and gain a deeper insight into their mechanisms.

IV. Models of non-heme monooxygenases

1. Mononuclear models of non-heme monooxygenases

The systems in which alkane hydroxylation is concomitant with the oxidation of low-valent metals by molecular oxygen can be regarded as the simplest functional models of non-heme monooxygenases. The concomitant oxidation of cyclohexane in systems with $FeCl_2$ and $SnCl_2$ in acetonitrile, which is a rather efficient process, can serve as an example.[202–204] The yield of the alcohol (calculated with respect to the metal) can be increased by lowering the concentration of the complex. Under optimum conditions, it can reach 20% with $SnCl_2$ and 30% with $FeCl_2$. The system with $SnCl_2$ manifests high selectivity (under certain conditions the alcohol is formed as the only product). Thus with an excess of the substrate, the $SnCl_2 : O_2 : ROH$ ratio is 1 : 1 : 1, *i.e.*, it is the same as for the monooxygenase reaction. However, a detailed kinetic analysis has shown that this reaction proceeds *via* a branched radical-chain mechanism, which involves the $^{\bullet}OH$ radical.[1]

A great number of analogous systems including simple salts ($FeCl_2$, $FeBr_2$, FeI_2, $MnCl_2$, $MnBr_2$, VCl_2, $CuCl_2$, $NiCl_2$) in which cyclohexane is oxidised to give the alcohol and the ketone, have been described; the yields are nearly identical for different salts.[205] The oxidation of cyclohexene does not yield the epoxide, cyclohex-2-enol being the main product. The regioselectivity of hydroxylation of 2-methylbutane (*i.e.*, the ratio of yields of the tertiary, secondary, and primary alcohols formed, is in accord with the regularity that is characteristic of the $^{\bullet}OH$ radical: $3^{\circ}/2^{\circ}/1^{\circ} = 13 : 5 : 1$. Toluene is oxidised to both benzyl alcohol and isomeric cresols (predominantly the *ortho*- and *para*-isomers). These systems are usually characterised by a low value of KIE in the oxidation of C_6H_{12} and C_6D_{12},[202] which is also typical of the hydroxyl radical.

One of the few proofs of the participation of non-radical intermediates in such systems has been found in the study of alkane oxidation in the $Ph_2Sn–O_2$ system.[206] Partial stereospecificity (20%) observed in the oxidation of *trans*-dimethylcyclohexane is suggestive of the oxenoid reaction that occurs as a background to the radical process. It has been assumed [206] that the peroxo complex Ph_2SnO_2 is the reactive intermediate in this reaction.

The system discovered in 1954 by Udenfriend *et al.*[207] was one of the first functional models of non-heme monooxygenases based on iron complexes. This system was made up of ascorbic acid and an Fe^{II} complex with ethylenediaminetetraacetate (EDTA) and could hydroxylate aromatic compounds by molecular oxygen. Later, Hamilton [88] found that this system could also hydroxylate cyclohexane (although in low yield) and epoxidise cyclohexene. In further studies, numerous systems containing non-heme complexes of bi- and tri-valent iron together with oxygen and a reducing agent or a source of active oxygen in various solvents have been proposed and analysed (Table 7).

As can be seen from Table 7, these systems simulate many reactions of atomic oxygen transfer catalysed by monooxygenases, such as aliphatic and aromatic hydroxylation, oxidation of heteroatoms, and epoxidation of alkenes. It is also evident that the characteristics of model systems (selectivity, isotope effect) strongly depend on the nature of the ligand, the source of active oxygen, and the presence of water.

Peroxides or molecular oxygen as oxidants together with various reducing agents are generally used in non-heme models. There is little or no data on the non-heme complexes (other than with cyclam [214] and cyclidene [197]) that can hydroxylate alkanes by atomic oxygen donors, such as iodosobenzene. Peroxides as oxidants simulate more completely molecular oxygen activation by monooxygenases including the stages of peroxo complex formation and O–O bond cleavage. However, difficulties emerge because free radicals generated simultaneously with the ferryl species initiate radical-chain oxidation.[217] Most papers devoted to this problem are aimed at the search for new ways of estimating the non-radical contribution to the overall oxidation.

One first proof of the non-radical hydroxylation catalysed by iron salts was found in cyclohexanol oxidation by hydrogen peroxide in the presence of $Fe^{II}(ClO_4)_2$ in acetonitrile.[208] It was found that the main oxidation product was *cis*-cyclohexane-1,3-diol obtained in a more than 70% yield. Such selectivity was attributed to the formation of a complex between the active oxidation intermediate, $Fe^{IV}=O$, and the OH group of the original alcohol. The nearest C–H bond, which is accessible for the attack, is that at the C(3) atom. Later, it was shown that this system can hydroxylate methane ($k_H/k_D = 5$) and ethane and oxidise dimethyl sulfoxide to dimethyl sulfone with a selectivity of about 96%.[209, 218] The data obtained have led to a conclusion about the involvement of oxoferryl in the hydroxylation of light alkanes at a low (below 1.5 M) concentration of H_2O_2 in solution.

It has been found that the selectivity of hydrocarbon oxidation concomitant with the activation of H_2O_2 on iron complexes in

Table 7. Effects of non-heme mononuclear models of monooxygenases.

Substrate	Catalyst	Oxidant	Products [a]	Ratio	KIE	Yield per oxidiser (%)	Ref.
Cyclohexanol (**5**)	$Fe(ClO_4)_2$	H_2O_2	*cis*-cyclohexane-1,3-diol + other alcohols	7 : 3	1.18		208
Methane	$Fe(ClO_4)_3$	H_2O_2	$CH_3OH + CH_2O$		5		209, 218
Dimethyl sulfoxide	$Fe(ClO_4)_3$	H_2O_2	Me_2SO_2			96 [b]	209, 218
Adamantane (**8**)	$[Fe(MeCN)_6](ClO_4)_3$	$NaIO_4$	**10**, (**12** + **9**)	1 : 24			210
Cyclohexane (**4**)	$FeCl_3$	H_2O_2	**5**, (**6** + **7**)	3 : 1	2.9 and 11	40 [c]	211
	$Fe(bipy)_2^{2+}$ (see [d])	Bu^tOOH	**5**, **6**, $Bu^tOOC_6H_{11}$	1 : 15 : 4	see [e]	40 [c]	211
	$Fe(cyclam)(OTf)_2$ [f]	H_2O_2	$C_6H_{11}OOH$, **5**, **6**	25 : 5 : 2	2.5 [c]	8–10 [c]	214
	$Fe(cyclam)(OTf)_2$	PhIO	**5**, **6**	2.5 : 1	4.3 [c]	8–10 [c]	214
	$[Fe(TPA)Br_2]ClO_4$ [g]	Bu^tOOH	**5**, ($C_6H_{11}Br$ + **6**), $Bu^tOOC_6H_{11}$	9 : 13 : 9	5.4	36 [c]	216
	$[Fe(TPA)Br_2]ClO_4$	MCPBA [h]	**5**, ($C_6H_{11}Br$ + **6**)	20 : 9		26 [c]	216
Cyclohexene	$FeCl_3$	H_2O_2	epoxycyclohexane, (allyl alcohols + ketone)	1 : 2.5		41 [c]	212
	$Fe(cyclam)(OTf)_2$	H_2O_2	the same	13 : 1		30 and 3	214
	$Fe(cyclam)(OTf)_2$	PhIO	"	14 : 1		30 and 3	215
n-Hexane	$Fe(cyclam)(OTf)_2$	H_2O_2	C(1)/C(2)/C(3) [i]	1 : 6 : 7			214
	$Fe(cyclam)(OTf)_2$	PhIO	the same	1 : 14 : 18			214
PhOMe	$FeCl_3-PC-Py$ [j]	O_2–Ascorbic acid	*o*-, *m*-, *p*-methoxyphenols	66 : 1 : 33		96 [k]	213

[a] See Scheme 1; [b] with respect to the DMSO; [c] overall product; [d] bipy is 2,2′-bipyridyl; [e] KIE 4.8, > 10.5, 5.4, respectively; [f] cyclam is 1,4,8,11-tetraazacyclotetradecane; [g] TPA is tris(2-pyridylmethyl)amine; [h] MCPBA is 3-ClC_6H_4COOOH; [i] C(1)/C(2)/C(3), relative yield of 1-, 2-, and 3-hexanols normalised per the C–H bond; [j] PC is $Py \cdot CrO_3$; [k] with respect to Fe.

aprotic solvents [219, 220] differs markedly from the selectivity of the radical-chain oxidation initiated by $^{\bullet}OH$ radicals.[202] This can be accounted for by the possibility of heterolytic cleavage of hydrogen peroxide with the formation of high-valent iron oxo-complexes, $Fe^{IV}=O$ and $Fe^{V}=O$, as the active oxidation intermediates.

The system based on the Fe^{II} complex with 1,4,8,11-tetraazacyclotetradecane (cyclam) and H_2O_2 (Table 7) is specific in that it can effectively epoxidise alkenes and hydroxylate alkanes.[214] It was suggested that this system contained an iron peroxo-complex stabilised by a hydrogen bond with the nitrogen of the ligand as the reactive epoxidising agent.

Various tripodal ligands have been used to control the coordination sphere of iron [216] (Table 7). By applying Me_2S as the oxene trap, Leising *et al.*[216] have demonstrated that the formation of the alcohol and the ketone in the cyclohexane oxidation by *tert*-butyl hydroperoxide occurs *via* a pathway which is different from that operating in the formation of dialkyl peroxide: the former two are apparently generated through an oxoferryl intermediate, while the latter is formed under the action of radicals. The results obtained earlier for heme models were also confirmed: electron-donor ligands at the Fe atom favour heterolytic dissociation of the peroxide and provide the stabilisation of the high-valent state of iron in the oxo-complex.

2. The Gif systems

The so-called Gif systems (named after the French town Gif-sur-Yvette) the first variant of which was proposed by Barton *et al.*[221] can also be related to the above-described model systems. In the decade that followed, these systems were intensely investigated,[222–226] and several versions were proposed (Table 8).

In all these versions, the Gif systems had a similar composition analogous to that of the above-mentioned mononuclear models. A characteristic feature of these systems is that they employ a pyridine–carboxylic (usually, acetic) acid mixture in various proportions (as a rule, 10 : 1) as the solvent. The majority of Gif systems utilise iron complexes as the catalyst with various ligands that strongly influence the rate of the reaction. Thus, the addition of picolinic acid to $FeCl_3 \cdot 6H_2O$ increases the reaction rate by a factor of tens, whereas pyridine-2-phosphoric acid fully inhibits this process.[239]

Table 8. Gif systems.

System [a]	Catalyst	Reducing agent	Oxidant	Ref.
Gif^{III}	Fe(II)/Fe(III)	Fe^0 (powder)	O_2	221
Gif^{IV}	Fe(II)/Fe(III)	Zn^0 (powder)	O_2	235, 236
GO	Fe(II)/Fe(III)	Hg (cathode)	O_2	237, 238
$GOAgg^{I}$	Fe(II)/Fe(III)		KO_2/Ar	239
$GOAgg^{II}$	Fe(II)/Fe(III)		H_2O_2/Ar	240, 241
$GOAgg^{III}$	Fe(II)/Fe(III)/ picolinic acid		H_2O_2/Ar	242
$GOChAgg^0$	Cu(I)/Cu(II)	Cu^0 (powder)	O_2	243
$GOChAgg^1$	Cu(I)/Cu(II)		H_2O_2/Ar	227

[a] The nomenclature of the Gif systems was proposed by Barton.[223]

The Gif systems have attracted the attention of investigators owing to their unusual selectivity in alkane oxidation. It was believed at first that secondary C–H bonds are oxidised more efficiently than tertiary ones (the $2^{\circ}/3^{\circ}$ ratio in the oxidation of adamantane reached 22, and for *trans*-decalin it was 36), the ketone being the main product. Later, it was found that in addition to the oxidation products, tertiary adamantyl radicals also yield coupling products with pyridine the content of which increases with a decrease in the oxygen concentration. At the same time, secondary adamantyl radicals do not form coupling products with pyridine irrespective of the oxygen concentration.[227] The regioselectivity of adamantane oxidation in these systems is $2^{\circ}/3^{\circ} = 1.0 : 1.1$ taking into account coupling products. Although the tertiary C–H bond does react several times faster than the secondary one, the selectivity of this reaction still differs markedly from that observed in ordinary radical processes.

Gif reactions can be conducted in *tert*-butyl alcohol and even in water at the corresponding pH values.[228–230] The predominance of a ketone among the reaction products, which is characteristic of the Gif systems, is determined by the solvent

(pyridine–acetic acid), which plays the role of a buffer by maintaining the pH at 6.5 which favours the catalytic dehydration of alkyl hydroperoxide to ketone.

The oxidation of alkyl-aromatic compounds in Gif systems yields the corresponding aldehydes and the ketones. The oxidation involves predominantly the secondary C–H bonds, but not the aromatic ring.[231] In the case of terminal disubstituted alkenes, the cleavage of the C=C bond yields a ketone and formaldehyde.[232] The presence of readily oxidisable substrates, such as alcohols, thiols, PPh_3, *etc.*, does not produce any inhibitory effect on the alkane oxidation in these systems.[228, 233, 243]

The detection of an alkyl hydroperoxide as an intermediate product of the cyclohexane oxidation was an important stage in the study of the mechanism of action of the Gif systems.[234] In these systems, cyclohexyl hydroperoxide is nearly completely converted into cyclohexanone (the ketone/alcohol ratio reaches 10). Alkyl hydroperoxides are intermediate products in the alkane oxidation in all the versions of the Gif systems including copper-containing ones.[243] Experiments with $^{18}O_2$ have shown that it was molecular oxygen (but not H_2O_2) that was the donor of the oxygen atoms in the alkyl hydroperoxide.[228]

It is natural to suppose that alkyl radicals are the precursor of hydroperoxides. This assumption has been confirmed by experiments with radical traps (Ph_2Se_2, Ph_2S_2, CCl_4, *etc.*), which generate the corresponding monosubstituted alkanes instead of the ketone.[234, 244] At low pressure (down to 40–10 mm Hg), where the concentration of O_2 and the yields of the oxidation products are minimal, a paramagnetic trap, 2,2,6,6-tetramethylpiperidine-*N*-oxyl (TEMPO), yields the corresponding derivatives with secondary and tertiary adamantyl radicals, the yield and the selectivity index (2°/3°) being the same as those for the oxidation products at normal pressure.[228]

At the same time, some experimental data, particularly the absence of coupling products of secondary adamantyl radicals with pyridine even at low concentrations of O_2 is a serious argument against the generation of free secondary alkyl radicals. Some other data are also in contradiction with the formation of free-radical alkyl intermediates.[245, 246]

Barton *et al.*[229] have interpreted the data concerning the selectivity of Gif systems in a framework of a reaction mechanism that included the generation of a complex of highly oxidised iron with oxygen, $Fe^{V}=O$. Its reaction with the secondary C–H bonds of an alkane gives a metal-alkyl complex, HO–Fe–R, with an Fe–C bond. The insertion of molecular oxygen or other reagents into this bond yields an alkyl hydroperoxide or different alkane derivatives. However, it was understood that this hypothesis fails to explain the whole body of evidence obtained with the Gif systems, particularly the lack of an obvious dependence of the reaction parameters on the nature of the metal.

Sawyer *et al.*,[220, 247] who paid much attention to the study of hydrogen peroxide activation on metal complexes suggested that such oxidative intermediates as $LFe^{II}OOH$ and $LFe^{IV}(OH)OOH$ (the latter is formed when the peroxide is in an excess over iron), which interact with alkanes to give intermediate complexes with Fe–C or FeOO–C bonds, can be generated in Gif and related systems. The simultaneous presence of radical and non-radical reactive intermediates in this reaction is also possible.

3. Functional models of methane monooxygenase

The selective oxidation of methane is a most serious problem because of the high strength of its C–H bond. Very few chemical systems known thus far can oxidise methane to methanol simulating enzymic oxidation under mild conditions.

The chlorides of some metals can promote hydroxylation of light alkanes concomitant with autooxidation in organic solvents.[248]

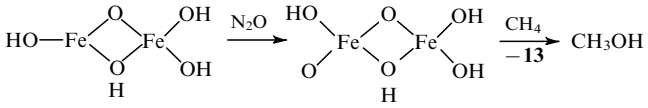
Recently, several systems for methane oxidation by hydrogen peroxide in trifluroacetic acid under relatively mild conditions have been proposed. Methyl trifluoroacetate is formed in the presence of Pd(II) and can further be hydrolysed to methanol.[249]

$$CH_4 + H_2O_2 \xrightarrow[2.\ H_2O,\ H^+]{1.\ Pd(II),\ CF_3COOH} CH_3OH\ .$$

Catalysis by Ru(II)[250] gives methanol and formaldehyde in a 4:1 ratio. These systems can catalyse the transfer of the oxygen atom from H_2O_2 to the C–H bond of methane, although metal-complex activation of the methane itself should not be ruled out.

A system for methane and ethane oxidation by oxygen in aqueous media in the presence of a metal catalyst (Pd/C) and a reducing agent (CO)[251] for which 1000 catalytic cycles were obtained at 85–100 °C is an interesting example of a heterogeneous analogue of methane monooxygenase (MMO). In this system, ethane is oxidised to CH_3CO_2H, HCO_2H, and CH_3CH_2OH in the 221:50:15 ratio. It is assumed that the $^{\bullet}OH$ radical does not take part in a hydrocarbon oxidation despite the intermediate formation of hydrogen peroxide.

Apparently, one of the best functional models of MMO is the oxidation of methane, carbon monoxide, and benzene by nitrous oxide on the so-called α-centres of iron-containing zeolites, ZSM-5.[251] These centres result from iron release from the crystalline lattice of the zeolite upon thermal treatment. Their nature still remains obscure. It is believed[252, 253] that they represent surface iron hydroxide clusters.[13] The dissociation of N_2O on α-centres results in the addition of the oxygen atom to yield the active oxidant **14**, which oxidises methane stoichiometrically at room temperature:



13 **14**

The use of functional models has made it possible to carry out mild homogeneous oxidation of methane; however, structural factors, such as the binuclear nature of the active centre of MMO, have not been taken into consideration.

4. Binuclear model systems

Binuclear iron complexes with one μ-oxo- or μ-hydroxo-bridge of the $(L_nFe)_2O(H)$ type are the simplest models of the active centre of MMO.[254]

One of the first attempts to use a binuclear μ-oxo complex, iron hydro(tris-1-pyrazolyl)borate, $\{Fe[HB(pz)_3]\}_2O$, in alkane hydroxylation by oxygen in the presence of a reducing agent (zinc) and an effector (acetic acid in CH_2Cl_2) was undertaken in 1988.[255] This work did not raise the problem of the preservation of the binuclear structure during functioning of the complex. However, further studies have shown that most of the binuclear complexes with a single μ-oxo bridge were unstable in the presence of reducing agents.[254] Later, Kitajima *et al.*[256] improved this system by introducing an additional stabilising ligand (hexafluoroacetylacetonate) into the binuclear complex; the excess of the ligand simultaneously fulfilled the role of an effector instead of acetic acid.

The $Fe_2O(DTBC)_2Cl_4$ complex, where DTBC is di(*tert*-butyl)catechol, catalyses the oxidation of alkanes (including methane) by molecular oxygen in the presence of a reducing agent (hydrazobenzene).[257] The binuclear complex proved to be stable under reaction conditions. The mononuclear complex is one order of magnitude less active than the binuclear one. The change in the regioselectivity of the hexane and adamantane oxidation as the binuclear complex is substituted for the mononuclear one shows that the iron complex is involved in the reaction with alkanes. It is concluded from these data that the binuclear oxoferryl intermediate plays the role of the active species. However, the structure of the hypothetical intermediate remains obscure.

The catalytic activity of a series of complexes of the $[Fe_2OL_4X_2](ClO_4)_2$ type, where L is 2,2′-dipyridyl (bipy), 4,4′-dimethyl-2,2′-dipyridyl or phenyl, and X is a labile ligand (H_2O,

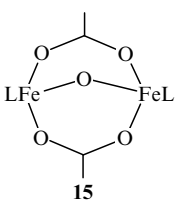
Cl^-, $CH_3CO_2^-$, $CF_3CO_2^-$), was studied in the reaction of alkane oxidation by peroxides.[258, 259] It was shown that the efficiency of these complexes depends on the lability of the ligand X. The rate of the reaction drops with an increase in the basicity of the ligand L. The best results in the cyclohexane oxidation by *tert*-butyl hydroperoxide (the yield of the oxidation products is > 50%, the reaction rate is 17 cycles per min) were obtained for the $Fe_2O(bipy)_4(H_2O)_2(ClO_4)_4$ complex in alkane oxidation (involving methane) by peroxides. The catalytic activity of the binuclear iron complex, $[Fe_2OL_4(H_2O)](ClO_4)_4$, was found to depend on the nature of the substituent in the ligand.[260] The dependence of the selectivity of hydroxylation of a series of alkanes and epoxidation of limonene on the nature of the ligand substituent and the effect of oxygen on the selectivity and the yield of the reaction products demonstrated that these processes occur not only by a radical mechanism, but also by a molecular mechanism, *i.e.*, with the transfer of the oxygen atom from the peroxide to the hydrocarbon mediated by the metal complex.[261]

Cyclohexanol is the single product of cyclohexane oxidation by *tert*-butyl hydroperoxide catalysed by $[Fe_2O(TPA)_2(H_2O)_2]^{4+}$, where TPA is tris(2-pyridylmethyl)amine, under conditions where the concentration of the hydroperoxide exceeds that of the catalyst by more than one order of magnitude. The peroxo complex $Fe^{III}OOR$, which can oxidise alkanes and the excess of the hydroperoxide by initiating radical-chain oxidation, has been assumed to be the reactive intermediate. The relative contribution of each of these mechanisms is determined by the concentration of the peroxide.[262]

Cyclohexane is also oxidised in high yield but at a much lower rate in the presence of two-bridge complexes of the $(L_nFe)_2O(OAc)$ type with μ-oxo- and carboxylate (OAc) bridges.[258, 263] The reactive intermediate in alkane oxidation by *tert*-butyl hydroperoxide in MeCN involving the binuclear complex, $[Fe_2(TPA)_2O(OAc)](ClO_4)_3$, was shown to be a metallocomplex compound. The substitution of TPA in the complex for analogous tetradentate ligands alters the KIE in the alcohol formation reaction, which provides direct evidence in favour of the involvement of the iron complex in the transfer of oxygen. Addition of Me_2S completely inhibits the formation of the alcohol and the ketone without any effect on diperoxide production. It is suggested[258] that the alcohol and the ketone are formed by the oxenoid mechanism, whereas the diperoxide is formed by the radical-chain reaction. The KIE of the diperoxide formation ($k_H/k_D = 7.7$) does not depend on the nature of the complex used. This fact is an additional proof that the metal complex intermediate does not take part in the diperoxide formation.[264, 265]

Three-bridge binuclear complexes structurally closely resemble the active centre of MMO. The first models **15** containing the nucleus, $(LFe)_2O(OAc)_2$, were developed by Wieghardt *et al.*[266] in Germany and by Lippard *et al.*[267] in the USA who employed terminal tridentate ligands.

A number of analogous complexes with different terminal and bridge ligands have been obtained. All these complexes efficiently simulate the spectral and magnetic properties of hemerythrine and ribonucleotide reductase (but not MMO), but do not possess any functional activity due to the lack of vacant sites for O_2 binding in their coordination spheres.



15

L is $C_6H_{15}N_2$,[266] 1,4,7-triazacyclononane;[266] $HB\left(N\overset{N}{\underset{}{\diagup\!\!\diagdown}}\right)_3^-$.[267]

The methods for preparing iron binuclear complexes with labile coordination sites have been elaborated in the past decade; some of these are based on the self-assembly from simple molecules:[268–270]

$$2FeOH^{2+} + 2L + 2RCOO^- \xrightarrow{Solv} [Fe_2OL_2(RCOO)_2(Solv)_2]^{2+} + H_2O$$

Solv is the solvent molecule.

This reaction can yield binuclear or tetranuclear complexes depending on the conditions.

The catalytic activity of such three bridge binuclear complexes in alkane oxidation is usually low.[271] The oxidation of methane and other alkanes by hydrogen peroxide catalysed by $[Fe_2O(bipy)_2(OBz)_2Solv_2](ClO_4)_2$ (where OBz is benzoate and Solv is H_2O or MeOH) in acetonitrile has been studied.[270] The solvent molecule in these complexes represents a labile ligand and can apparently be substituted by H_2O_2. The KIE for the oxidation of C_6H_{12} and C_6D_{12} ($k_H/k_D = 3.0$), the selectivity of hexane oxidation, and the 5/6 parameter for cyclopentane and cyclohexane oxidation suggest the involvement of the Fe=O formed by the splitting of the FeOOH intermediate. It has been shown that such complexes with a labile coordination sphere are more active in alkane oxidation than complexes with a closed coordination sphere.

In a recent study[272] it has been shown that the $[LFe_2O(OAc)_2](ClO_4)_2$ complex, where L is the tetradentate hexapyridine ligand, efficiently catalyses alkane oxidation by *m*-chloroperbenzoic acid. If dichloromethane and dibromomethane were used as the solvents, the products of oxidation of cyclohexane (**4**) contained chlorocyclohexane (**7**) and bromocyclohexane, respectively. Radical traps completely inhibited the oxidation. These results together with the regioselectivity data and the KIE led the authors[272] to conclude that alkane oxidation in this system occurs *via* a radical mechanism with the formation of an alkyl radical.

As new information about reactive intermediates of the catalytic cycle of binuclear monooxygenases became available, attempts were undertaken to synthesise the corresponding model compounds.[273–276]

Early studies on simulation of peroxo intermediates were based on the reactions of binuclear complexes of trivalent iron with hydrogen peroxide.[274–276] Later, a series of Fe_2^{II} complexes with an open coordination sphere and the ability to bind O_2 have been synthesised.[273] The ligands able to bind two metal centres that have been prepared on the basis of 1,3-diaminopropan-2-ol and the carboxylate bridge provide the appropriate ligand environment (N_3O_2) for each Fe^{II} atom. In addition, one molecule of water is sometimes coordinated to one of them. Reversible[277] and irreversible[278, 279] binding of molecular oxygen to these and related complexes is observed at low temperatures. An increase in temperature results in the formation of oxidised Fe_2^{III} (see Refs 278 and 279). The oxygen adducts have been characterised as (μ-1,2-peroxo)Fe_2^{III} complexes on the basis of their Raman and Mössbauer spectra.

The factors that enhance the stability of peroxo complexes have been established. They include the presence of bulky substituents in the tetradentate ligand that attenuate the electron-donor properties of nitrogen and form simultaneously a hydrophobic environment for the peroxo-centre.[277, 278] The peroxo-intermediate, $Fe^{3+}-O_2^{2-}-Fe^{3+}$ on the basis of the $[Fe_2(Ph\text{-}bimp)(C_6H_5COO)](BF_4)_2$ complex (where Ph-bimp is 2,6-bis-{bis[(1-methyl-4,5-diphenylimidazol-2-yl)methyl]aminomethyl}-4-methylphenoxide) is stable even at room temperature.[280] The introduction of donor substituents into the bridge ligand accelerates the decomposition of the peroxo complexes. This is accounted for by an increase in the electron density at the metal, which facilitates the cleavage of the O–O bonds and simultaneously stabilises the highly oxidised state.[273] The stabilising effect of polar solvents on peroxo intermediates has also been reported.[277, 279, 280]

The parameters of the optical and the Raman spectra of model peroxo complexes are in good agreement with the corresponding parameters of the intermediate P formed in the catalytic cycle of MMO.[76, 281]

The catalytic systems based on binuclear iron complexes are rather unstable. In the course of the reaction, such complexes either decompose into mononuclear complexes or are destroyed due to ligand oxidation. Moreover, reductive activation of O_2 can be accompanied by nonproductive reactions between the oxidised and reduced forms of the iron complexes, which decreases the activity of the homogeneous systems. Immobilisation of complexes on solid supports favours their stabilisation and decelerates nonproductive bimolecular reactions.

5. Immobilised binuclear complexes

The synthesis of an iron complex on the surface of silica gel modified by imidazole derivatives has been reported.[282, 283] The structure of a binuclear complex has been proposed on the basis of UV, EPR, and Mössbauer spectroscopic data. This heterogeneous catalyst induces the oxidation of methane and other alkanes by molecular oxygen at room temperature in the presence of a reducing agent (zinc dust), an effector (acetic acid), and an electron carrier (methyl viologen) in acetonitrile. The regioselectivity of hexane oxidation suggests that the $^{\bullet}OH$ radical is the reactive species in this system. A recent report[284] deals with cyclohexane oxidation by an analogous heterogeneous complex which, in contrast to the former, has two μ-oxo bridges and a dimethylamino group instead of imidazole, but no carboxylate bridges. This complex effectively hydroxylates cyclohexane (57 cycles per catalyst), but does not oxidise methane at an appreciable rate.

6. The mechanism of alkane oxidation and reactive intermediates in non-heme model systems

Fish *et al.*[285, 286] have concluded that alkane oxidation in all the systems having a composition $LFe^{II/III} + H_2O_2 - O_2$ yield an alkyl hydroperoxide or its complex with iron, FeOOR, as the intermediate product, which, in turn, points to the formation of the substrate-derived radical $R^{\bullet}$ as the intermediate. This conclusion was confirmed by the detection of diperoxides in the systems based on mono- and bi-nuclear iron complexes with *tert*-butyl hydroperoxide as the oxidant.[262, 287]

Evidence that alkane oxidation in the

$[Fe_2O(bipy)_4(H_2O)_2](ClO_4)_4 - H_2O_2$,[261] $[Fe_2O(tmima)(OAc)_2](ClO_4)_2 - H_2O_2$[286]

[tmima is tris(1-methylimidazol-2-yl)amine],

$[Fe_2O(TPA)_2(H_2O)_2]^{4+} - Bu^tOOH$, and

$[Fe(TPA)Cl_2]^{+} - Bu^tOOH$[272]

systems involves alkyl radicals is provided by the dependence of the concentration and composition of oxidation products on the concentration of molecular oxygen in the reaction medium.

Presumably, radical oxidation is initiated by the oxygen radicals generated either by activation of peroxides on metal complexes[288, 289] or in the oxidation of the excess of peroxide by oxenoid intermediates.[156, 262] The possibility of generation of the alkyl radicals $R^{\bullet}$ through detachment of hydrogen from the hydrocarbon by reactive peroxo- or oxo-iron complexes has also been considered.[252, 272, 286] Redox potentials for the formation of radicals from hydrocarbons with the general formula $HCH_{3-n}(CH_3)_n$ varies from 1.60 to 2.25 eV;[194] therefore, the detachment of H by oxenoid intermediates occurs apparently only in the oxidation of the less stable (tertiary or activated) C—H bonds.

Although short-living oxenoid intermediates have been detected in systems with non-heme iron, they have not been adequately characterised. The intermediate $Fe^{IV}=O$ has been effectively generated by laser photolysis of a Fe^{II}salen complex (salen is 1,2-disalycylideneaminoethane) with molecular oxygen.[290] It was shown that iron complexes with EDTA, like their porphyrin analogues, can transfer an oxygen atom from peroxy acids and peroxides by splitting the latter *via* a heterolytic mechanism.[291] The formation of a non-heme ferryl complex has been observed in aqueous solutions by spectral methods.[292]

The ability of binuclear Fe^{II} complexes with 1,2-bis(2-hydroxybenzamido)benzene to transfer the oxygen atom from a peroxy acid and PhIO to cyclohexene *via* the intermediate formation of Fe=O has been established.[293, 294] In the absence of the substrate, the spectroscopically identified Fe=O intermediate (its lifetime is 1.5 h at −78 °C) was converted into the μ-oxo complex.

A reactive oxidative intermediate generated in the reaction of a binuclear iron μ-oxo complex, $[Fe_2O(TPA)_2](ClO_4)_4$, with H_2O_2, in a system that hydroxylates alkanes and epoxidises alkenes, has been characterised in the most detail.[295] Analysis of the EPR, Mössbauer, and Raman spectra, together with the data from the elementary and mass-spectrometric analyses,[296] suggest that the reactive intermediate has an $Fe_2(\mu\text{-}O)_2$ nucleus with a delocalised electron. In its characteristics it is similar to the reactive intermediate **X** in the catalytic cycle of ribonucleotide reductase.[296]

Obviously, these are only the first steps on the way to the simulation of non-heme monooxygenases. On the whole, this problem is still far from being finally solved. There are relatively few systems for which the involvement of well-characterised binuclear complexes in alkane oxidation has been confirmed experimentally. There are practically no reliable data on the nature of the reactive intermediates. At the same time, the experimental results altogether suggest that the construction of efficient analogues of MMO is a task of the nearest future. One important problem on the way is the stabilisation of binuclear complexes during the catalytic cycle. Preliminary data show that a major breakthrough can be expected from the application of complexes immobilised on solid supports. A strong impetus for the construction of adequate models of MMO is the X-ray determination of MMO structure. However, the design of efficient structural and functional analogues will also require great skills of synthetic chemists because the modelling of the protein environment of the active centre is fraught with considerable difficulties. Apparently, the design and synthesis of more complicated models that reproduce, with a great degree of accuracy, the structure of the iron complex and its environment in the protein globule will play a prominent role in both the simulation of cytochrome P-450 and the construction of analogues of non-heme monooxygenases.

V. Conclusion

The results presented in this review allow one to make a conclusion about the increasing interest in the enzymic oxidation of alkanes and the construction of chemical models of these reactions. Special interest of investigators has recently been focussed on the relatively poorly investigated enzyme, methane monooxygenase, as well as on the biomimetic systems based on non-heme binuclear iron complexes. Studies of cytochrome P-450 and its chemical models for which many fundamental problems still remain unsolved are also very important.

Undoubted progress in the field of natural and biomimetic monooxygenase systems should be mentioned. The use of modern physical methods permits the prediction, with an increasing degree of reliability, of the structure of reactive species involved in the transfer of the oxygen atom to the substrate. The formulation of a hypothesis about the intermediate formation of species with pentacoordinated carbon during the insertion of the oxygen atom into the C—H bond has been important for the understanding of the mechanism of monooxygenation. This hypothesis, which allows prediction of the quantitative distribution of the oxidation products in some cases has eliminated the contradictions evolved in the analysis of the two earlier proposed mechanisms, namely, the oxenoid mehanism, which entails direct

insertion of the O atom into the C—H bond, and the cryptoradical (oxygen rebound) mechanism.

The progress in the chemical simulation of monooxygenases demonstrates that novel efficient, highly selective catalytic systems for alkane oxidation will hopefully be created based on them in the nearest future.

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