Structural-functional modeling of non-heme oxygenases

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The nature designed metal-containing oxygenases to perform the most difficult oxidation reactions, such as the alkane oxidation, including the oxidation of methane, the most inert alkane. The enzymatic oxygenation, which proceeds under physiological conditions, *i.e.*, in the absence of severe chemical treatment, is characterized by high efficiency and selectivity as yet unattainable in chemical processes. A search for chemical systems reproducing the prominent features of oxygenases is based on the structural-functional modeling of the active sites of these biocatalysts. Recent studies have shown that it is, in principle, possible to design biomimetic catalysts having as good characteristics as biocatalysts.

Key words: biomimetic oxidation, oxygenase modeling, non-heme oxygenases, iron complexes, selective hydrocarbon oxidation, structural-functional modeling.

The ability of some iron-containing enzymes to catalyze the chemo-, regio-, and stereoselective oxidation of nonactivated hydrocarbons gave impetus to the search for the corresponding biomimetic catalysts.[1,2] Studies of the heme-containing enzyme cytochrome P450 and its models showed that the high catalytic activity of iron-containing oxygenases is due to the fact that the enzymatic oxidation results in the generation of short-lived intermediates, *viz.*, high-valent iron-oxo complexes referred to as ferryl intermediates (Fe=O), which can oxidize various substrates, including alkanes.[3—5] These intermediates have high reactivity comparable with that of free oxygen atoms and hydroxyl radicals. In recent years, it has been shown that precursors of ferryl intermediates, *viz.*, peroxide intermediates, can oxidize certain substrates as well; however, their activity is much lower than that of ferryl intermediates.[5]

High selectivity of metalloenzymes is achieved through a hierarchical assembly starting from metal ions and their nearest coordination environment, which includes both fixed endogenous ligands bound to the polypeptide backbone and exchangeable ligands that mark labile coordination sites necessary for the catalysis. The present review deals with the modeling of these structural units referred to as active sites although this term often includes also the second coordination sphere of the metal complex and the adjacent hydrophobic cavity necessary for the binding of the hydrocarbon substrate.

Oxygenases catalyze the oxygen transfer from molecular oxygen to the substrate *via* ferryl and peroxide intermediates to form alcohols in the case of alkanes and to give epoxides and diols in the case of olefins.[1,2] Oxygenases catalyzing the transfer of one oxygen atom to the substrate, as in the case of the formation of alcohols from alkanes or epoxides from olefins, are called monooxygenases. Dioxygenases catalyze the transfer of both oxygen atoms of molecular oxygen to the substrate, for example, in the oxidation of olefins to diols. The activation of molecular oxygen requires the transfer of electrons to it from a particular reducing agent. In the absence of reducing agents, hydrogen peroxide and other peroxide compounds, *N*-oxides, and hypohalide compounds can serve as sources of active oxygen species for oxygenases (usually, in the ferri form), which substantially simplifies the functional modeling of these enzymes.

The present review is restricted to publications on the modeling of binuclear and mononuclear non-heme iron-containing oxygenases with consideration for a breakthrough in the understanding of the structure and the mechanism of action of these enzymes achieved in the last decade.

Models of the active site of the soluble form of methane monooxygenase

The only iron-containing oxygenase capable of oxidizing methane, the so-called methane monooxygenase (sMMO), contains two carboxylate-bridged iron atoms in the active site[6,7] (Fig. 1). In addition to the endogenous nitrogen and oxygen ligands bound to the polypeptide backbone, there are also labile hydroxyl ligands and a coordinated water molecule, whose role remains unclear. Since the coordinated water molecule is retained throughout the catalytic cycle, its role is probably to stabilize oxygen intermediates, as was suggested based on quantum

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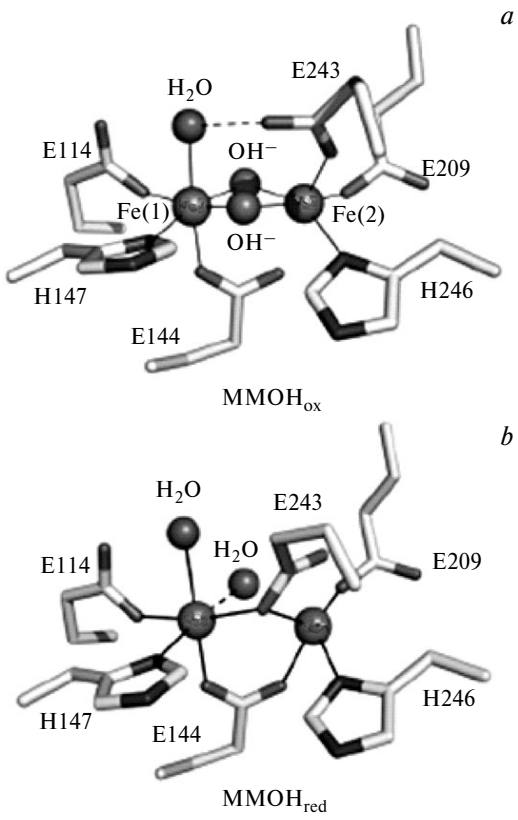
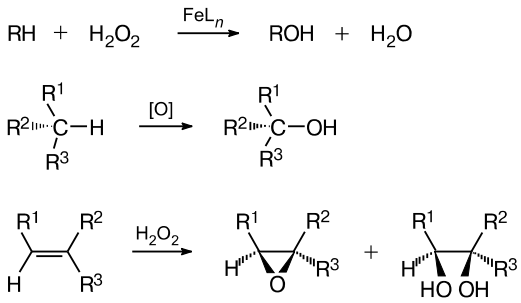


Fig. 1. Active site of sMMO in the oxidized, ferri, (*a*) and reduced, ferro, forms (*b*).

chemical calculations.[8] Along with the surprising ability to activate the strongest C—H bond, sMMO has the unusual and, at the same time, highest selectivity, preferring methane to other, less inert, hydrocarbons and oxidizing methane exclusively to methanol.

In early attempts to model sMMO, our research group[4,9] and other teams[10,11] used binuclear iron μ-oxo-μ-carboxylate complexes. In particular, we have studied the oxidation of methane and other alkanes by hydrogen peroxide catalyzed by the new complex $[Fe_2O(bpy)_2(OBz)_2(H_2O)_2](ClO_4)_2$ (bpy is bipyridyl) in aqueous acetonitrile.[12,13] An increase in the yield of alcohols after the addition of $HClO_4$ and a considerable isotope effect $k_H/k_D = 3.1$ were accounted for by the heterolytic cleavage of coordinated peroxide to form the active ferryl intermediate that attacks alkane. To verify this hypothesis, the simplest binuclear complexes containing labile coordination sites of the general formula $[Fe_2O(L)_4(H_2O)_2](ClO_4)_4$ (L is bpy, 4,4′-Me_2bpy, 4,4′-$(ClCH_2)_2$bpy, phenanthroline (phen), or 5-NO_2phen) were synthesized and their catalytic activity in the oxidation of methane and other alkanes was investigated.[14] The dependence of the catalytic activity in the oxidation of methane and cyclohexane on the nature of the substituent in the ligand, the retention of the configuration of the asymmetric tertiary carbon atom rather than the racemization during the oxidation of *cis*- or *trans*-1,2-dimethylcyclohexane, the fact that the cyclopropane ring remains primarily intact during the oxidation of *trans*-1-methyl-2-phenylcyclopropane (the formation of the long-lived cyclopropylmethyl radical would lead to the cyclopropane ring opening), and the formation of epoxides as the major products of the oxidation of olefinic compounds instead of allylic oxidation products (Scheme 1) are evidence of the contribution of the two-electron (rather than the usual radical-chain) mechanism of the oxygenation.[15,16] These studies have shown that the presence of labile coordination sites at the iron atom play an important role in the catalysis. It should be noted that, due to the possible reversible hydrolysis in aqueous acetonitrile solutions, $Fe^{III}—O—Fe^{III} + H_2O \rightleftarrows 2\ Fe^{III}OH$, in the case of simple complexes it is difficult to conclude whether the mononuclear or binuclear complex is responsible for the catalysis regardless of the initial state.

Scheme 1



The retention of the configuration in the course of the hydroxylation of the C—H bond is well known for heme models of cytochrome P450 and is evidence of the nonradical mechanism of the oxidation.[17] Along with other results, this proved that the μ-oxo-iron complexes $[Fe_2OL_4(H_2O)_2](ClO_4)_4$ catalyze the stereospecific transfer of an oxygen atom from hydrogen peroxide to alkanes and olefins (see Scheme 1). This was the first observation of the stereospecific oxygenation of alkanes by non-heme models, and it confirmed that these reactions are similar to the enzymatic oxygenation of nonactivated C—H and C=C bonds *via* ferryl intermediates.

The mechanism of the biomimetic oxidation by hydrogen peroxide was studied in more detail by researchers from the University of Minnesota[2,18,19] using the mononuclear iron complex with the tetradentate nitrogen-containing ligand tripicolylamine (tpa) as an example. These researchers were the first to "capture" the peroxide and ferryl intermediates at low temperatures and characterize them by different spectroscopic methods. In Russia, studies in this field are performed by Professor Talsi in the G. K. Boreskov Institute of Catalysis of the Siberian

Branch of the Russian Academy of Sciences, where the perferryl intermediate Fe^{V}=O was detected in these systems for the first time by ESR spectroscopy.[20] Earlier, the involvement of ferryl intermediates in the oxygen transfer has been also supported by the ^{18}O incorporation from $H_2{}^{18}O$ that was added to the reaction mixture into the reaction product due to the fast exchange of the ferryl oxygen with water.

The catalysis of the hydroxylation of nonactivated C—H bonds and the epoxidation of double bonds by model complexes containing nitrogen donors is generally related to the involvement of perferryl intermediates in the $Fe^{III}Fe^{V}$ catalytic cycle[18—21] shown in Scheme 2.

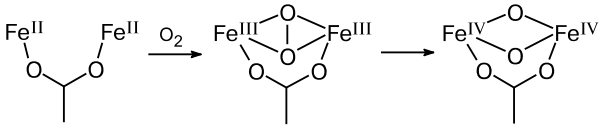
Scheme 2

$$Fe^{III} + H_2O_2 \longrightarrow Fe^{III}OOH \longrightarrow Fe^{V}{=}O + RH \longrightarrow Fe^{III} + ROH$$

The formation of the hydroperoxide intermediate in MeCN at −40 °C was reliably established by different methods.[22] Since the $Fe^{III}OOH$ intermediate cannot activate stable C—H bonds, it was suggested that its heterolytic cleavage leads to the perferryl intermediate, which is an active oxidant. Recently,[20] the oxoperferryl intermediate Fe^{V}=O has been identified by ESR spectroscopy at −70 °C in the model system Fe(tpa)—MeCN—H_2O_2.

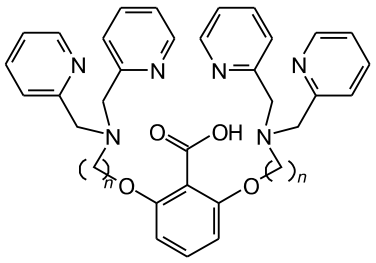
The modeling of the binuclear active site of sMMO is a difficult problem because neither the exact structure of active intermediates nor the detailed mechanism of the activation of O_2 and the hydrocarbon oxygenation are currently known. In 1995, I suggested the bridging mechanism[23] for the activation of O_2 by sMMO (Scheme 3) as an alternative to the commonly accepted terminal mechanism analogous to the mechanism of action of cytochrome P450.[24] According to the new mechanism,[23,25—27] the activation of O_2 occurs with the involvement of both iron atoms and does not require protons, as in the case of cytochrome P450. It is suggested that a μ-1,2-peroxide intermediate or an isomeric bridged intermediate with an unusual structure is initially formed (see Scheme 3). The latter is transformed into a new active intermediate, *viz.*, high-valent iron bis-μ-oxo complex (diferryl complex), through homolytic cleavage of the peroxide O—O bond, and this intermediate can, presumably, attack methane containing the strongest C—H bonds. More recently, this mechanism was experimentally[28,29] and theoretically[30,31] supported. It should be noted that evidence for the involvement of protons in the catalytic cycle of sMMO has been recently reported, but the mechanism of this involvement differs from the mechanism for cytochrome P450.[32] The bridging mechanism requires two labile coordination sites at each iron atom. We took into account this fact when designing new ligands and complexes. We also took into consideration the fact that complexes based on simple ligands are often labile in solution and, consequently, they not always adequately model the well-arranged structure of the active site of the enzyme.

Scheme 3



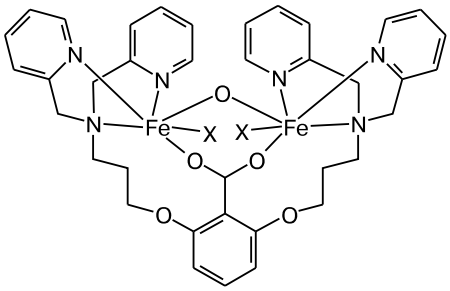
With the aim of constructing the structural model of the active site of sMMO and stabilizing binuclear complexes in solution, the tripodal dinucleating polydentate ligands LH 2,6-$[(PyCH_2)_2N(CH_2)_nO]_2C_6H_3COOH$ (**1**, **2**) containing the immobilized carboxylate moiety,[33—37] which are distant analogs of the polypeptide backbone of the metalloenzyme, were designed, and the binuclear iron(III) μ-carboxylate complexes $[Fe_2OL(X)_2]_2[ClO_4]_m$ (**3**, m = 2, 3) were synthesized.[33—35] These complexes serve as models of the oxidized (ferri) form of sMMO (see Fig. 1, *a*). Each octadentate ligand binds two iron atoms, and the remaining vacancies are occupied by labile ligands X, such as ions of the exogenous carboxylate group or water molecules.

According to the X-ray diffraction data, in the crystalline state complex **3b** (2X = $PhCOO^-$) exists as a dimer (Fig. 2), which dissociates to monomeric binuclear complexes in solutions containing a catalyst (a catalytic solution).[33—35]



1, **2**

n = 2 (**1**), 3 (**2**)



3a,**b**

X = H_2O (**3a**), 2X = $PhCOO^-$ (**3b**)

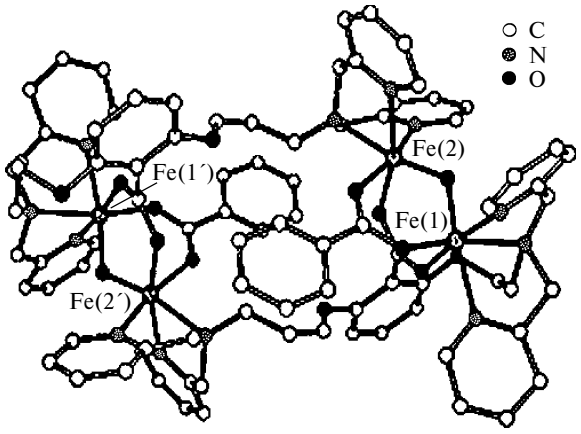
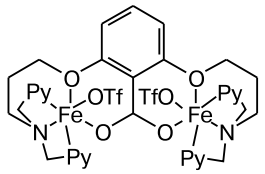


Fig. 2. X-ray crystal structure of complex **3**.

The presence of labile coordination sites (O^{2-} and X) in the orientation suitable for the catalysis is an important structural unit of the complexes. These complexes catalyze the oxidation of methane to methanol by hydrogen peroxide at room or slightly elevated temperatures (the turnover number of the catalyst TON = 2—7 at 20—60 °C), and, in experiments with cyclohexane and *cis*-1,2-dimethylcyclohexane, they show features of the monooxygenase mechanisms of the alkane oxidation, in particular, the ^{18}O incorporation from labeled water into the product and the stereoselectivity of the process.[35—37] Studies by electrospray ionization mass spectrometry (ESI MS) showed that the binuclear structure is retained up to micromolar concentrations of the complexes in the catalytic solution due to the preorganized tripodal ligand. Hence, it can be suggested that binuclear bridging peroxide that is initially formed and occupies the labile coordination sites is then transformed into the binuclear ferryl intermediate. This conclusion is confirmed by the fact that the oxygen label from labeled water added to the reaction mixture is present in the resulting alcohol. Taking into account the structural similarity between the complexes and the active site of sMMO, their ability to oxidize methane under ambient conditions, and the evidence of the involvement of the ferryl intermediate, these complexes can be considered as structural-functional models of sMMO, although it is more correct to consider them as the first step to these models. Since the efficiency of oxygenation was low, we suggested that this fact is associated with an insufficient stability of the active complex in solution due to the flexibility of the tripodal ligand in use.

Taking into account the fact that in the nature sMMO is functionally active in the reduced form, we synthesized the iron(II) complex $[Fe^{II}_2L(OTf)_2](OTf)$ (**4**) with tripodal ligand **2**, which can be considered as the possible model of the reduced form of sMMO, and studied its reactions with O_2 and H_2O_2 in the presence and in the absence of alkanes.*

* The paper is going to be published in *Izv. Akad. Nauk*, *Ser. Khim* [*Russ. Chem. Bull., Int. Ed.*], 2011, No. 10.



4

However, according to the X-ray diffraction data, the formation of complex **4** is accompanied not by the expected formation of the second bridge between the iron atoms with the involvement of the auxiliary ligand (H_2O, HO^-, or TfO^-) but by the coordination of the ester oxygen atoms of the ligand L to the iron atom and the change in the conformation of the carboxylate from the typical *syn*,*syn*, like in complex **3** and in the active site of sMMO, to the relatively rare *anti*,*anti* conformation. This leads to an elongation of the Fe···Fe distance with the result that the bridging peroxide intermediate necessary for the activation of molecular oxygen by these models cannot be formed. Due to this structure, complex **4**, unlike complex **3**, reproduces neither the structural nor functional features of the active site of sMMO, and it does not catalyze the oxidation of alkanes by hydrogen peroxide or O_2. Apparently, this fact may be responsible for low activity of complex **3**, because the initially active complexes that are formed in solution can be transformed into inactive complexes due to an insufficient rigidity of the tripodal ligand. Alternative approaches to the modeling of the active site of sMMO were published in the literature,[6,38] but the efficient biomimetic oxidation of methane was achieved in none of the cases. Nevertheless, with certain stipulations, let us note the highly selective, but only *stoichiometric* (TON = 1), oxidation of methane to methanol by nitrous oxide (N_2O) at room temperature in the presence of surface iron complexes,[39,40] which are formed after the thermal treatment of zeolite FeZSM-5. Finally, the catalytic oxidation of methane by hydrogen peroxide in water to give mainly CH_2O and HCOOH at 25—60 °C in the presence of the binuclear phthalocyanine iron complex containing the $Fe^{III}—N=Fe^{IV}$ moiety has been recently reported.[41] The maximum turnover number of the catalyst, which was achieved in this reaction, was 438. The yield was 30—50% based on hydrogen peroxide. The spectroscopic and isotopic studies confirmed that this reaction proceeds *via* the peroxide and ferryl ($O=Fe^{V}—N=Fe^{IV}$) intermediates. However, the theoretical calculations indicate that this complex is similar to cytochrome P450-type heme-containing oxygenases.[42]

Models of mononuclear non-heme oxygenases

The active site of mononuclear non-heme oxygenases (Fig. 3) contains the octahedral iron complex, one face of which is occupied by three endogenous ligands, *i.e.*, two imidazole rings of histidines and one carboxylate, forming

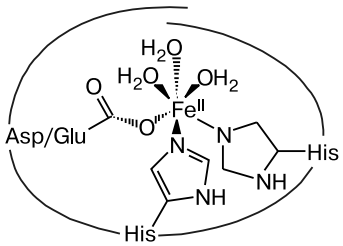


Fig. 3. Scheme of the active site of mononuclear N,N,O oxygenases.

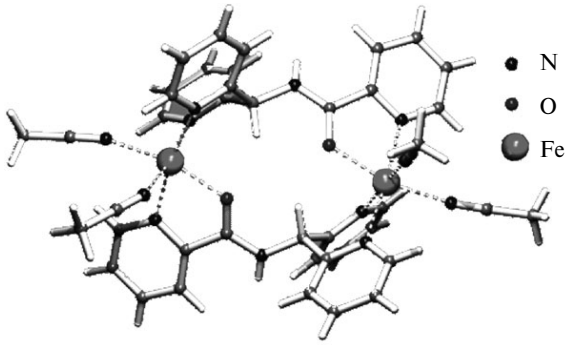


Fig. 4. X-ray crystal structure of complex **5**.

the so-called N,N,O-facial triad, and the opposite vertices are occupied by labile ligands, generally, by water molecules.[43] These three adjacent labile coordination sites provide an ideal platform for catalysis, enabling the simultaneous coordination and the mutual orientation of oxygen, substrate, and cofactor molecules in anyone combination.

The earlier models of non-heme oxygenases proposed by our[4,9] and other research teams[10,11] contained only nitrogen donors. With the aim of obtaining a more adequate model of the active site structures for these oxygenases containing, along with nitrogen donors, also endogenous oxygen donors, we synthesized the tetradentate tripyridylcarboxamide ligand, *N*-[bis(2-pyridyl)methyl]-pyridine-2-carboxamide ($Py_2CHNHCOPy$, tpcaH), whose carbonyl oxygen atom can be coordinated to the iron atom.[44] The reaction of the iron(II) salt with one equivalent of this ligand afforded the dimeric complex $[Fe^{II}(tpcaH)]_2^{4+}$ (**5**), in which, according to the X-ray diffraction data,* each tpcaH ligand is coordinated to the iron atom through the N atoms of all three pyridine moieties and the carbonyl O atom and forms a bridge between two iron atoms (Fig. 4). As opposed to the dimeric structure (D) of the complex in the crystalline state, in an acetonitrile solution the complex exists, as was shown by ESI MS, ^1H NMR spectroscopy and quantum chemical calculations, in equilibrium with two monomers with different structures, $[Fe^{II}(tpca)(MeCN)_2]^{2+}$ (M_1) and $[Fe^{II}(tpca)(MeCN)_3]^{2+}$ (M_2) (Fig. 5). Complex **5** catalyzes the selective and stereospecific oxidation of saturated hydrocarbons by hydrogen peroxide presumably *via* the perferryl intermediate. The latter is generated from M_2 and has the N,N,O-facial coordination of the potentially tetradentate tpcaH ligand serving as the structural model of the 2-histidine-1-carboxylate facial triad of non-heme oxygenases. Compared to other biomimetic complexes, the new carboxamide complex is more stable in the catalytic solution, less prone to the nonproductive decomposition of hydrogen peroxide to O_2 and water and, correspondingly, is most efficient in the alkane oxidation.

Table 1 compares the characteristics of the alkane oxidation by model iron complexes with phen, tpa, and tpcaH ligands: the turnover number (TON), the ratio of the concentrations of alcohol and ketone ([A]/[K]), and the stereospecificity of the processes expressed as the degree of retention of the configuration (RC) of the asymmetric carbon atom after the oxidation of *cis*-1,2-dimethylcyclohexane (DMCH).

To reproduce the unique N,N,O-facial triad characteristic of the active sites of non-heme mononuclear oxygenases, the tridentate dipyridylcarboxamide ligand *N*-[bis-(2-pyridyl)methyl]benzamide (dpcaH) was synthesized and then the iron(II) complex $[Fe(dpcaH)_2](OTf)_2$ (**6**) was prepared.[45] The crystal structure of the latter is shown in Fig. 6. The new complex contains two tridentate ligands. However, in spite of the coordinative saturation, complex **6** proved to be catalytically active in the olefin oxidation. This is associated with the fact that in the catalytic solution (according to the ESI MS data), one of the ligands completely dissociates to form the 1 : 1 complex with the facially coordinated tridentate N,N,O ligand and three labile coordination sites, which is apparently structurally similar to the active site of the enzyme. Complex **6** proved to be the most efficient biomimetic complex for the selective *cis*-dihydroxylation of various olefins, being a good structural-functional model of NNO dioxygenases. It is essential that this process occurs with 99% stereoselectivity, which characterizes the oxidation as the true *cis*-di-

Table 1. Oxidation of cyclohexane and *cis*-1,2-dimethylcyclohexane (DMCH) by hydrogen peroxide catalyzed by model iron complexes

Complex	Cyclohexane		DMCH RC* (%)
	TON	[A]/[K]	
Fe(phen)[16]	14	3	90
Fe(tpa)[18]	3	5	99
Fe(tpcaH)[44]	50	7	96

* The degree of retention of the configuration RC (%) = $= 100 \cdot (A - B)/(A + B)$, where A is the yield of the product with retention of the configuration of the starting hydrocarbon, and B is the yield of the racemate.

* The paper is going to be published in *Izv. Akad. Nauk, Ser. Khim* [*Russ. Chem. Bull., Int. Ed.*], 2011, No. 10.

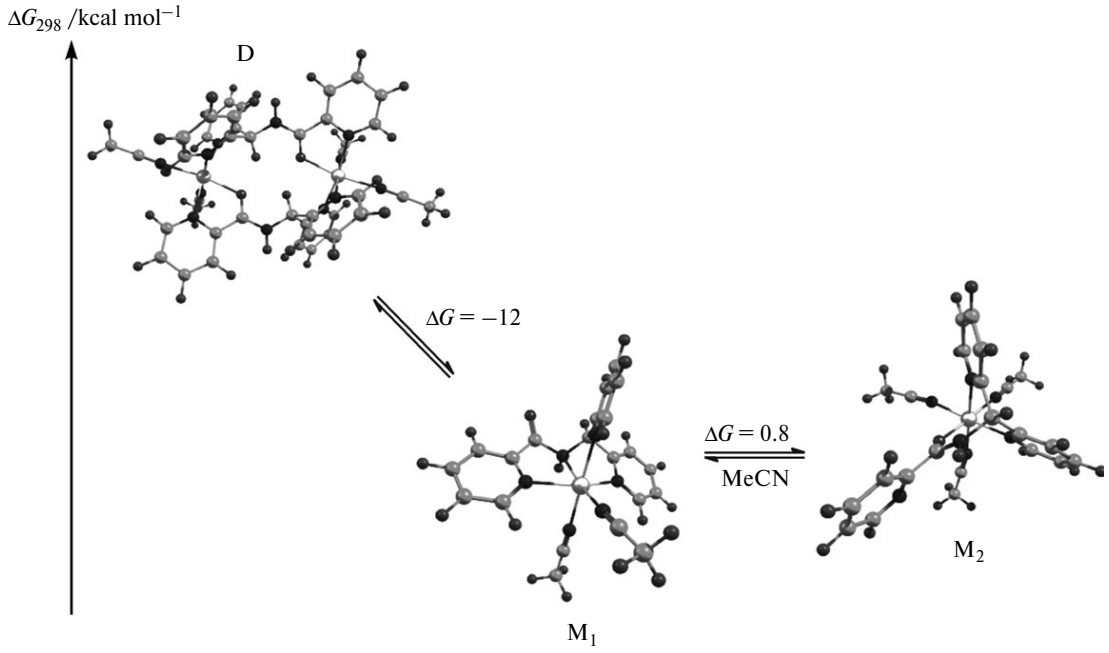


Fig. 5. Thermodynamics of complex iron(II) ions of complex **5** in a solution in MeCN calculated by the DFT method.

hydroxylation. Thus, the dihydroxylation of *cis*- and *trans*-hept-2-enes afforded *cis*- and *trans*-heptane-2,3-diols, respectively. The observed ^{18}O incorporation from $H_2{}^{18}O$ into the reaction product in the course of dihydroxylation catalyzed by complex **6** confirms that one of the ligands in this complex completely dissociates in solution, and this fact is attributed to the possible formation of the peroxide intermediate containing, along with peroxide coordinated in a bidentate fashion, a water molecule. Evidently, the mixing of oxygen isotopes is possible due to the easy proton transfer in the resulting active intermediate *cis*-$Fe^{IV}(OH)_2(^{18}OH_2)$ (see below).

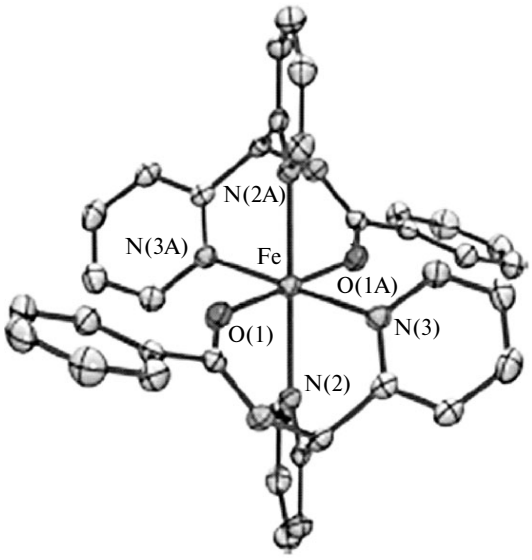


Fig. 6. X-ray crystal structure of complex **6**.

The study of the catalytical mechanism of the *cis*-dihydroxylation of alkenes by complex **6** using isotope methods led to the conclusion[46] that the reaction proceeds through the $Fe^{II}Fe^{IV}$ catalytic cycle shown in Scheme 4, where the coordinated solvent molecules (MeCN and H_2O) are omitted for simplicity.

Scheme 4

$$Fe^{II} + H_2O_2 \longrightarrow \eta_2\text{-}Fe^{II} \cdot H_2O_2 \longrightarrow$$
$$\longrightarrow cis\text{-}Fe^{IV}(OH)_2 + C{=}C \longrightarrow Fe^{II} + C(OH){-}C(OH)$$

The involvement of the *cis*-dihydroxyferryl intermediate in the *cis*-dihydroxylation of olefins has been postulated earlier for another iron(II) complex based on the results of quantum chemical calculations.[47] The compound η_2-$Fe^{II} \cdot H_2O_2$ is the precursor of this intermediate. The formation of this compound requires the presence of two adjacent labile donors in the coordination sphere of the complex, which facilitates the homolytic mechanism of the O—O bond cleavage in hydrogen peroxide.

With the aim of obtaining the precise structural model of the active site of naphthalene dioxygenase, researchers from the University of Minnesota[48] with our participation synthesized the tripodal ligand containing two pyridyl moieties and one carboxylate group, whose conformation is favorable for the formation of the mononuclear iron(II) complex (complex **7**). Like the active site of the enzyme,

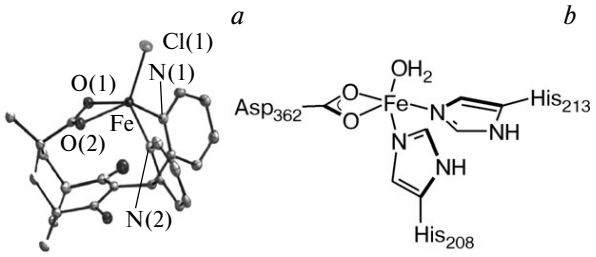


Fig. 7. X-ray crystal structure of complex **7** (*a*) and the scheme of the active site of naphthalene dioxygenase (*b*).

this model complex, according to the X-ray diffraction data (Fig. 7), is five-coordinate and contains, along with two N ligands, the carboxylate group in the bidentate coordination mode and a chloride anion as a monodentate exchangeable ligand. However, complex **7** does not assist in the oxidation by hydrogen peroxide because of insufficient lability of the chloride ligand. The replacement of the chloride ion by the more labile trifluromethanesulfonate ligand results in the formation of a new complex, which, like the enzyme, exhibits high selectivity in performing the *cis*-dihydroxylation.

In the metal complex catalysis, the change in the structure of the ligand determines to a substantial degree the catalytic characteristics of the complex, revealing particular properties of the metal ion, which results in a great diversity of catalysts based on the same metal. The selectivity of $[Fe(dpcaH)_2]^{2+}$ (complex **6**) evaluated as the diol to epoxide ratio was in the range of 80—100 depending on the structure of alkene.[46] However, being a rather efficient catalyst for the oxidation of olefins, this complex is almost inefficient in the catalysis of the oxidation of C—H bonds, even such weak bonds as the allylic C—H bonds in cyclohexene.[45]

The reaction of 2 equiv. of the carboxamide ligand tpcaH with Fe^{II} produces the complex $[Fe^{II}(tpcaH)_2]^{2+}$ (complex **8**).* The crystal structure of complex **8** appeared to be similar to that of complex **6**. In complex **8**, the potentially tetradentate ligand $Py_2CHNHCOPy$ employs only its N,N,O-facial donor triad for coordination to iron, retaining the pyridyl moiety as the outer-sphere donor. This complex, like complex **6**, serves as a model of the family of mononuclear N,N,O oxygenases (see Fig. 3). As can be seen from Table 2, the introduction of the outer-sphere N donor (the pyridine moiety) into this complex (as a result of the replacement of the phenyl group by the pyridyl group in the carboxamide moiety of the ligand) dramatically changes the chemoselectivity of the complex catalyst. Complex **6** is a highly selective catalyst for the *cis*-dihydroxylation of olefins and is almost inefficient as the catalyst for the oxidation of even weak allylic C—H bonds of olefins,[45] whereas complex **8** does not catalyze the dihydroxylation of olefins but assits in their epoxidation and can abstract the H atom from nonactivated C—H bonds in the hydroxylation of cyclohexane. The observed chemoselectivity of complex **8** definitely corresponds to the $Fe^{III}Fe^{V}$-catalytic cycle (see Scheme 2).

How it can be explained that the introduction of the pyridyl moiety instead of the phenyl ring, which does not change the core of the complex and which has no substantial effect on its spectroscopic and structural characteristics, leads to such a substantial change in the chemoselectivity of the complex catalyst?

The change in the chemoselectivity means that the introduction of an outer-sphere donor either retards the formation of the key intermediate in the *cis*-dihydroxylation or facilitates the formation of the key intermediate in the epoxidation of olefins. In the case of complex **6**, the formation of the solvolytic complex containing three solvent molecules in *cis* positions with respect to each other is confirmed[45] by the ^{18}O isotope exchange between hydrogen peroxide and $H_2{}^{18}O$, which can occur in the intermediate $Fe^{IV}(OH)_2(OH_2)$ *via* proton transfer. On the one hand, the retardation of the formation of the intermediate η_2-$Fe^{II}\cdot H_2O_2$ (see Scheme 4) in the case of two labile *cis*-coordination sites may be associated with stabilization of the isomeric intermediate η_1-$Fe\cdot H_2O_2$, in which hy-

* The paper is submitted to *Russ. J. Inorg. Chem.*

Table 2. Oxygenation of cyclohexene (RH) by hydrogen peroxide under an argon atmosphere catalyzed by complexes **6** and **8**

Complex	TON				Conversion of H_2O_2 (%)	Reference
	Double bond		Allylic α-C—H bond			
	Epoxide	Diol	Cyclohexenol	Cyclohexenone		
6[a]	0.0	5.6	0.5	0.4	65	45
8[b]	4.5	0.1	14.0	7.0	51	—[c]

[a] The reaction conditions: Fe : H_2O_2 : RH = 1 : 10 : 1000, temperature was 25 °C, the reaction time was 1 h.
[b] The reaction conditions: Fe : H_2 : RH = 1 : 50 : 1000, temperature was 20 °C, the reaction time was 2 h.
[c] The paper is submitted to *Russ. J. Inorg. Chem.*

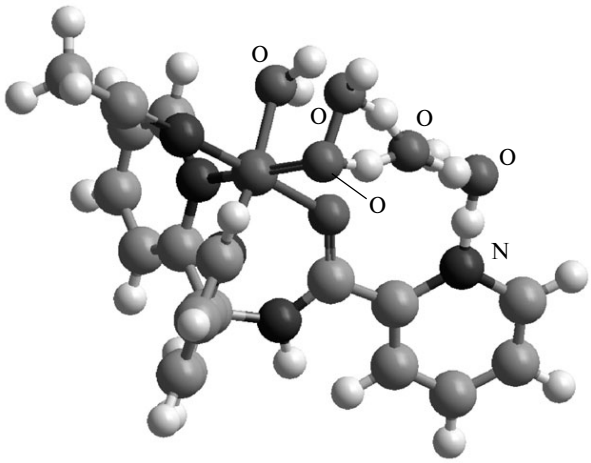


Fig. 8. Proposed stabilization of the linear configuration of the peroxide intermediate η_1-Fe(tpcaH)(H_2O_2)$(S)_2 \cdot nH_2O$ by a hydrogen bond chain *via* the involvement of several water molecules between the peroxide and the outer-sphere pyridyl donor ligand.

drogen peroxide occupies only one coordination site. This stabilization is possible due to the fact that η_1-Fe$\cdot H_2O_2$ is linked to the outer-sphere pyridyl moiety by a bridge consisting of several hydrogen-bonded water molecules (Fig. 8). In the presence of a water molecule in the *cis* position with respect to the coordinated peroxide, the peroxide bond cleavage may follow the heterolytic mechanism *via* proton transfer.[21] On the other hand, the heterolytic cleavage of the peroxide O—O bond in this structure may be more preferable than the homolytic cleavage due to the catalysis of the proton transfer from the water pool by the pyridyl moiety (see Fig. 8). This acid-base catalysis of the heterolytic cleavage of the peroxide bond was observed both in biological[49] and chemical systems.[50] The pyridyl moiety, which is not coordinated to the iron atom, serves as an outer-sphere donor and is, apparently, involved in the acid-base catalysis of the heterolytic cleavage of the peroxide bond to form the active perferryl intermediate, thus modeling the function of the outer-sphere amino-acid residues in the enzymatic oxidation. Although the exact mechanism is unknown, the presence of the outer-sphere donor dramatically changes the chemoselectivity of the metal complex catalyst by switching the olefin oxygenation from the *cis*-dihydroxylation to the epoxidation. It should be noted that the presence of the outer-sphere donor substantially increases the power of the catalyst enabling the hydroxylation of nonactivated C—H bonds. Apparently, this also occurs in the catalysis of the hydroxylation of alkanes by the iron complex with the potentially hexadentate monophenolate ligand $(PyCH_2)(CH_2COOH)N$-$CH_2C_6H_2(OH)(Me)CH_2N(Pr^i)CH_2Py$, which we have described recently.[51] This ligand is coordinated to the iron atom in a tetradentate fashion, due to which the $CH_2N(Pr^i)CH_2Py$ group remains free and can be involved in the catalysis as the outer-sphere donor. The important role of outer-sphere noncovalent interactions in the modulation of the strength of the active site was found both in the nature and in biomimetic model systems.[52,53]

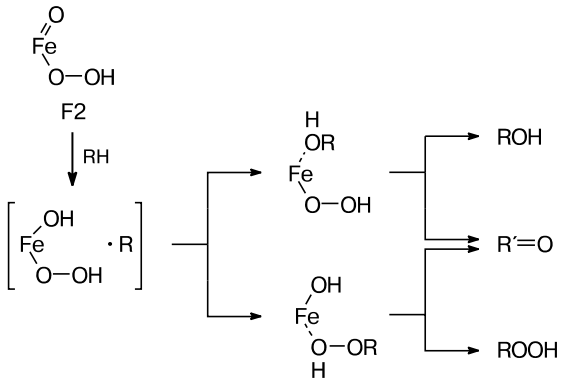
Let us revert to the oxygenation of alkanes by the model compounds of non-heme sites proposed earlier.[2,16,18,44] The endogenous tetradentate ligand (or two bidentate ligands) and two labile monodentate ligands exchangeable with hydrogen peroxide or water molecules is a common structural unit of these complexes. The catalysts synthesized by the two research groups ensure the stereospecificity of the process (*i.e.*, the almost 100% retention of the configuration of the oxidized alkane in the product). However, some differences in other characteristics of the oxidation, such as [A]/[K] and the ratio of the attack on the *tert*- and *sec*-C—H bonds of adamantane (3°/2°) suggesting different mechanisms of the oxygenation, remained unclear for a long time. Different procedures were used in the experiments on the oxidation of hydrocarbons: either the whole amount of hydrogen peroxide was introduced immediately in the beginning of the reaction together with other reagents,[16] or hydrogen peroxide was slowly added during the reaction with the use of a syringe pump to decrease the nonproductive decomposition of hydrogen peroxide in the catalase reaction.[18] In the latter case, both the yield based on H_2O_2 and the stereospecificity were higher. We supposed that a large difference in the steady-state concentrations of hydrogen peroxide in these experiments is one of the factors responsible for the differences in the parameters [A]/[K] and 3°/2°. To verify this supposition, we performed the oxidation of alkanes in the presence of several model complexes in two ways: with a gradual addition of a dilute H_2O_2 solution with a syringe during the reaction, as described in the study,[18] and with the addition of the whole amount of hydrogen peroxide in the beginning of the reaction, *i.e.*, at two—three orders higher concentrations.[54] A comparison of the results showed that a high degree of retention of the configuration of alkane in the resulting tertiary alcohol is observed in the oxygenation of *cis*-1,2-dimethylcyclohexane regardless of the H_2O_2 concentration. This confirms that in both cases, the reaction proceeds in the coordination sphere of the metal complex. However, the parameters [A]/[K] and 3°/2° are obviously decreased with increasing concentration of hydrogen peroxide. This is particularly pronounced in the case of the complex containing the strongest donor ligand. Although there is also the dependence on the electron-donating properties of the ligand, the influence of the H_2O_2 concentration is evident. The study of the changes in the parameter [A]/[K] for the iron carboxamide complex in a wider concentration range of hydrogen peroxide showed that the value of [A]/[K] after extrapolation to the zero H_2O_2 concentration is approximately four times larger than that after extrapolation to the infinitely large con-

centration. An analogous situation is observed also for the bond selectivity 3°/2°. Since these parameters characterize, in essence, the selectivity of the intermediate that attacks alkane, it is evident that the change in the hydrogen peroxide concentration leads to a change in the active intermediate. To explain these results, we hypothesized that at high concentrations of hydrogen peroxide, a new ferryl intermediate containing the peroxide group, Fe^{V}=O(OOH), is formed. We called this intermediate the ferryl peroxide intermediate (hereinafter F2, unlike the simple ferryl intermediate F1). The extrapolation to the zero and infinitely large concentrations of hydrogen peroxide gives the values of [A]/[K] and 3°/2° for the intermediates F1 and F2, which characterize the selectivity of both intermediates.

Parameter	F1	F2
3°/2°	16	3
[A]/[K]	4	1

It is seen that the ferryl peroxide intermediate F2 has lower selectivity and, correspondingly, higher activity than the ferryl intermediate F1. Scheme 5 shows the proposed mechanism of the alkane oxidation through the intermediate F2, similar to the oxygen rebound mechanism proposed earlier[55] for the ferryl intermediate. The hydrogen abstraction from alkane gives rise to a short-lived radical complex, in which the recombination of the alkyl radical can proceed through two channels: with the involvement of either hydroxyl or peroxyl groups. The decomposition of the resulting radical complexes gives the following three products: alcohol, ketone, and alkyl peroxide. The retention of the configuration is 98% after the oxygenation of the *tert*-bond in *cis*-1,2-dimethylcyclohexane and is 76% after the hydroperoxidation of this bond, as opposed to the radical chain oxidation, which always results in racemization.[56] The retention of the configuration in this mechanism is due to short lifetimes of the radical complexes. If the radical escapes from the solvent cage, it can undergo racemization, and in this case, the partial retention of the configuration will be observed; the longer the lifetime of the radical complex the smaller the degree of retention. This mechanism is supported by the observation of the formation of alkyl peroxide only at high concentrations of hydrogen peroxide and the fact that its formation is independent of the presence of oxygen. The most important evidence for the validity of the proposed mechanism is based on the fact that alkyl hydroperoxide is characterized by the relatively high degree of retention of the configuration of the attacked carbon atom in the starting alkane, which confirms that, in the case of model complexes, alkyl hydroperoxide, like alcohol, is formed in the coordination sphere of the metal complex without the involvement of free radicals.

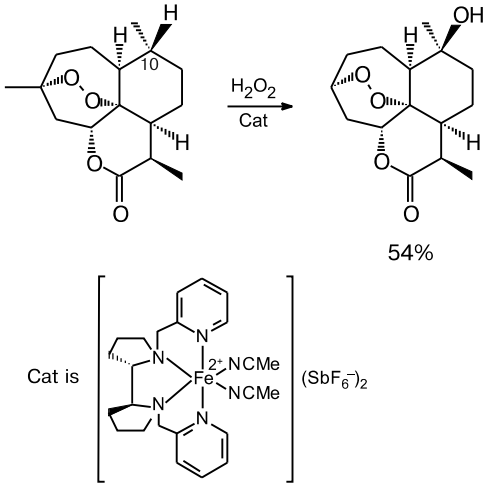
Scheme 5



Conclusions

The combined efforts of biologists and chemists in studies of the structures and the mechanism of action of natural oxygenases resulted in a considerable progress in the last decade. A deep insight into the enzymatic oxygenation opens prospects for the design of efficient and highly selective chemical catalysts and for the development of environmentally safe and cost effective chemical processes. Studies on the modeling of oxygenases laid the basis of the development of preparative methods for the selective oxidation of nonactivated C—H and C=C bonds in complex polyfunctional molecules. As an example, Scheme 6 shows the hydroxylation of artemisinin at the *tert*-C(10)—H bond. The molecule of this plant antimalarial drug used in ancient Chinese medicine contains numerous C—H bonds and sensitive functional groups. The use of a cheap oxidizing agent, *viz.*, hydrogen peroxide, and a biomimetic catalyst similar to those mentioned above enables the one-pot synthesis of the pure product in good yield (see Scheme 6),

Scheme 6



the stereochemistry and the labile functional groups being retained without the introduction of protecting or activating groups.[57]

Therefore, although the application of biomimetic catalysts for the functionalization of hydrocarbons on a multiton scale with the aim of more efficiently employing oil and gas raw materials is a distant prospect, these catalysts may be currently used for the design of innovative oxidation processes in fine organic synthesis and pharmaceutical chemistry.

To summarize, the above-considered studies showed that it is possible to design, based on the nature experience, biomimetic catalysts for the selective oxidation of hydrocarbons to alcohols, epoxides, and diols by the cheap and environmentally safe oxidant, *viz.*, hydrogen peroxide, according to the mechanisms through peroxide and ferryl intermediates with different structures.

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