CHEMISTRY & CHEMICAL TECHNOLOGY

Vol. 4, No. 1, 2010 Chemistry

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ENVIRONMENT FRIENDLY SPIN-CATALYSIS FOR DIOXYGEN ACTIVATION

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Received: July 04, 2009

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Abstract. Catalysis of controlled selective oxidation of hydrocarbons utilizing molecular oxygen, being of great potential for the environmental friendly chemical industry, has to be designed by analogy with biological enzymatic reactions. Catalysis of oxidation of hydrocarbons by paramagnetic dioxygen avoiding a classical free radical chain mechanism needs to overcome spin prohibition. Classification of spin catalysis in biological activation of dioxygen is presented in this review and discussed in connection with known industrial processes. A few important examples are highlighted to illustrate the role of spin effects in O_2 binding to myoglobin, glucose oxidases, cytochrome P450, horse reddish peroxidase, and non-heme irone complexes.

Keywords: ferric alkylperoxo complexes, hemoglobin, myoglobin, cytochrome P450, Fe(IV)-oxo compound I, glucose oxidase, spin-orbit coupling, high-spin – low-spin transition.

1. Introduction

Industrial interest in dioxygen chemistry has always been great because of free of cost air [1, 2]. It has even increased in the recent time since the latest insight into the mechanisms for the O_2 activation by enzymes [3-6] is promising for the green chemistry, which mimics natural catalysts. Selective catalytic oxidation of alkanes, alkenes and aromatic molecules is the most important reaction pathway for converting petroleum-based feedstock to special organic products in the high-yield chemical industry [1]. Catalytic use of molecular oxygen as a primary oxidant will necessitate the development of environment friendly "no-waste" technologies which will provide economically viable syntheses. The success of these technologies depends on the use of metal catalysts which mimic metalloenzymes [1-12]. Nature is the best catalyst engineer for oxidation of organic fuel (food) for respiration and metabolism. In biology a wide range of selective oxidations of hydrocarbons by molecular oxygen are catalyzed by oxygenases, peroxidases and cytochromes [13-47]. Just recently the Cherkasy region, together with the world scientific community, has celebrated the 150 year anniversary of the birthday of their country man, academician A.N. Bach. He was the first to introduce the idea of molecular oxygen activation during his studies of horse reddish peroxidase (HRP) and stressed the role of peroxides in biological oxidation [Ber. Deutsch. Chem. Gessel., 1903, **36**, 600-605]. Even nowadays the HRP is an object of the most intriguing discoveries, including magnetic field effects [42] and quantum chemistry calculations for electronic mechanism of the HRP catalytic cycle [4, 39, 43]. The HRP-catalyzed oxidation of NADH can be presented by the schemes [42]:

$$\begin{aligned} &HRP + NADH \rightarrow Per^{2+} + NADH^{+\bullet} \rightarrow \\ &Per^{2+} + O_2 \rightarrow Comp\ III + NAD^{\bullet} \rightarrow \\ &Comp\ I + NADH \rightarrow Comp\ II \end{aligned} \qquad (1)$$

where compound II represents the Fe(IV)=O group connected with the porphin ring, compound I represents Fe(IV)=O connected with the radical cation of porphin, and compound III represents the porphin-containing Fe(IV)–OOH group. Here porphin denotes the protoporphyrin IX linked with protein chain by the histidine residue. The scheme (1) is compatible with the magnetic-field dependence of the rate constants measured by stopped-flow spectroscopy [42]. The catalytic cycle starts with electron transfer from NADH to native HRP (Per^{3+}) to produce $NADH^{+\bullet}$ radical and the ferroperoxidase intermediate (Per^{2+}); (Per^{3+}) actually means here the ferric porphin-Fe(III). Interconversion of NADH to form NAD^+ occurs by hydride transfer, in which the H^+ ion and two electrons are transferred between the C(4) carbon atom of nicotineamide ring and substrate [44]. Catalytic cycle of NADH oxidation in the presence of hydrogen peroxide

begins with a two-electron oxidation of the HRP enzyme to form Fe^{IV} and the porphyrin cation radical; this compound I is a highly reactive species that can accept one electron from NADH to form a $NADH^{+\bullet}$ radical cation, which can undergo deprotonation to yield $NAD^{\bullet}$ and compound II [42]. The ferro-porphin intermediate (Per^{2+}) is known now to have a quintet ground state [45] as also follows from DFT calculations [4]. The modern data illustrate a complex nature of dioxygen activation and indicate the role of electron spin in such processes.

A common feature of many dioxygen activation processes is the involvement of multivalent transition metal ions [1], the most important of which are iron ions with high residual valence and a variety of possible spin states [17]. They enter the great family of heme proteins and related biopolymers [17-20]; the ferrous and ferric ions in this family of enzymes provide spin catalysis during dioxygen activation [18-27]. To mimic this spin catalysis in industrial chemical synthesis one needs to know the main principals of spin conservation in chemical reactions, quantum spin transitions, the role of exchange, and magnetic perturbations in electronic mechanisms of chemical transformations [26-38]. The key question in controlled oxidation of hydrocarbons is a spin-prohibition of direct reaction of dioxygen with diamagnetic molecules to give singlet state diamagnetic products [2]. Almost all chemically stable organic substances have an even number of electrons with electronic spins being paired, thus their total spin and magnetic moment are equal to zero [16]. The oxygen molecule is a famous exception to this general rule: unlike many chemically stable organic compounds the O_2 molecule has two unpaired electrons with parallel spins and the triplet ground state [1]. One electron occupies a $\pi_{g,x}$ antibonding molecular orbital (MO) and the other one occupies the other $\pi_{g,y}$ antibonding MO, whose axis is perpendicular to the former axis [3]. The two outer electrons in two degenerate $1\pi_g$–MOs provide the lowest triplet state of the type [↑][↓], where the quantum cells [][] denote the degenerate π_g-orbitals. These two unpaired electrons in antibonding π_g–orbitals are responsible for specific character of the dioxygen interaction with radicals (combustion) and chemically stable diamagnetic compounds (slow oxidation). Two antibonding π_g–vacancies makes it possible to transform dioxygen into O_2^- and O_2^{2-} anions, whose formation is strongly dependent on the presence of electron donors and magnetic perturbations that affect the spin prohibitions [3]. Such O_2 activation represented by spin catalysis is widely implemented in biosystems and could be simulated and used to convert hydrocarbons to ketones, alcohols and epoxides in chemical industry by consumption of dioxygen from the air.

In this review a general concept of nonadiabatic spin-transitions in enzymatic and engineered catalysis is presented in order to illustrate how various aspects of this complex interdisciplinary science work together to create environment friendly chemical technology of low cost in near future.

2. Spin-Prohibition of Dioxygen Reactions and Two Types of Spin Catalysis

Apparently simple O_2 molecule with its small size has attracted hundred years of research in biochemistry, metallurgy, organic and inorganic chemistry, atmospheric spectroscopy, catalysis, combustion, and photochemistry in order to understand the connections between chemical (electronic) structure and observed properties of dioxygen [4]. According to Hund rule, two unpaired electrons in two degenerate $\pi_{g,x}$- and $\pi_{g,y}$-orbitals have a lower repulsive energy in the triplet state compared to the singlet state. Because of this oxygen has intrinsic magnetic moment due to spins of two unpaired electrons (it is paramagnetic) and O_2 addition to organic compounds is spin forbidden: starting reactants have the total spin S = 1 (from the O_2), whereas the oxidation products are diamagnetic (S = 0) [12]. This is the reason why organic matter may exist in the oxygen-rich atmosphere.

After combustion study by Lavoisier and demise of the phlogiston theory, a new era of chemistry started. Following Berzelius idea of catalysis, several gas-phase catalytic oxidation processes were developed: Winkler process for Pt-catalyzed oxidation of SO_2 to SO_3 and the Ostwald process for oxidation of ammonia to nitric acid on platinum [1]. One of the first catalytic oxidation of hydrocarbons was the ethene to epoxide conversion on the Ag catalyst, discovered by Lefort in 1935. Then the free-radical chain theory of autoxidation was proposed by Emanuel *et al.* [3] and a number of catalytic oxidation processes were developed [1-7] which are based on a specific activation of the triplet O_2 molecule and spin-catalysis [3].

There are several ways to overcome spin prohibition in O_2 reaction with organic molecules. Combustion of organic fuels requires activation in the form of high-temperature ignition stage [12], *i.e.* generation of primary radicals. Similar initiation is necessary for autoxidation. Reaction of $R^{\bullet}$ radical (one unpaired electron, S = 1/2, doublet state) with O_2 molecule is spin-allowed, since the starting reactants ($O_2 + R^{\bullet}$) and product ($RO_2^{\bullet}$), respectively [↑][↑]+[↓] and [↑][↑↓] both have the doublet states, which provide the radical chain character of the combustion reactions [12]. Here the quantum cell [↑] denotes MO with α spin; spin pairing (↑↓) corresponds to a new chemical bond R–O. The radical $RO_2^{\bullet}$ can decompose into radical $RO^{\bullet}$ and biradical $^{\bullet}O^{\bullet}$ thus providing branching chain reaction. In radical chain combustion the energy is released

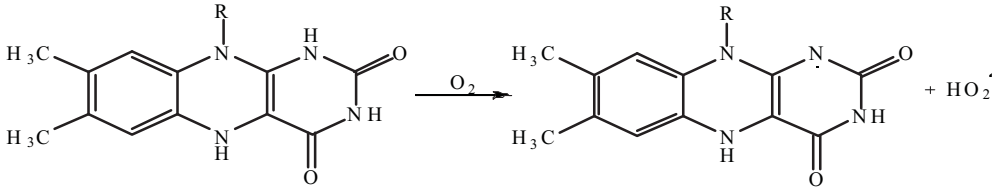


Fig. 1. Spin-allowed activation of dioxygen by reduced flavins [2]

in the form of heat and light without any specific control (until the fuel exhaustion or radical trapping by antioxidants). Such mechanism of oxidation by molecular oxygen can not be realized in living cells. Cells meet their energy needs in the course of metabolic processes using strictly controlled energy of oxidation of organic compounds in their reactions with dioxygen, overcoming spin prohibition without high-temperature ignition step of radical chain [9]. An aerobic life evolved due to specific kinetic prohibitions to reactions of paramagnetic oxygen with diamagnetic organic substances. The main reason for sluggish O_2 reactivity at the ambient conditions is the spin prohibition, namely, the starting reagents have two unpaired spins (from the O_2 molecule), while in diamagnetic oxidation products (CO_2, H_2O, N_2) all spins are always paired. Overcoming of this prohibition by generating radicals to interact with dioxygen (like in combustion) is inadmissible to living matter. Since the cells can not resist large temperature gradients, they have to transform the energy released through oxidation to some kind of chemical energy prior to dissipation in the form of heat. This occurs by combining oxidation with the ATP synthesis. All versatile energy-supplying metabolic processes and reactions occur under subtle enzymatic regulation, which is strictly spin-dependent [12, 18-23].

Reaction of triplet dioxygen with singlet molecule forming two radicals, each being in doublet state, is spin-allowed process; but usually it is a highly endotermic reaction [2]. In biochemistry the high endotermicity of the primary radical generation is usually considered [1] to be overcome at normal temperature only in the case of very reactive substrate, *e.g.* reduced flavins (Fig. 1), that form resonance-stabilized radicals.

This reaction (Fig. 1) is considered as a key step in the activation of dioxygen by flavin-dependent oxidases and oxygenases [1, 9]. If the subsequent biological reactions proceed by radical chain mechanism, no questions to the spin selection rule would arise. But bio-oxidation does not involve radical chain reactions; otherwise living organism would be burned. Apparently the reaction shown in Fig. 1 is a puzzle: the primary radical pair presented in such oxygenase is produced in the triplet state and the reaction can not be completed with production of stable products inside the enzyme active center without the triplet-singlet (T-S) transition [27]. In the following the mechanism of such T-S transition is explained by analysis of specific internal magnetic interaction, which represents an example of a particular (type I) spin catalysis [21, 28].

A second way to overcome spin prohibition (type II spin catalysis) in O_2 activation is the catalysis by paramagnetic transition metal ion (M^n): $O_2 + LM^n = LM^{n+1}–O–O^-$ [2, 21], where L denotes some ligands. The resulting metal-dioxygen complex is expected to react selectively with organic molecules at moderate temperature [1, 7]. This expectation gave rise to the extensive search for selective catalysts for "dream reactions" (epoxidation, hydroxylation of hydrocarbons) [1, 2] and was based on comparison with enzymatic reactions which include metallo-proteins, like cytochromes, peroxidases, copperaminooxidases, *etc.* [12-31]. Multivalent and paramagnetic character of the transition metal ions was considered as an obvious reason for the search [1, 2], but the role of spin effects in O_2 activation was not stressed so far [27, 32-41]. The non-zero spin of the metal ion (for example, S = 2 for ferrous ion, or S = 1/2 for ferric ion) in reaction with O_2 (S = 1) can provide a number of possible spin states; thus a diamagnetic hydroxylation product can be obtained either on a high-spin or low-spin potential energy surface. The spin-prohibition is removed here together with the potential barrier lowering by involvement of the so-called exchange interaction [3, 21, 27]. This formally denoted type II spin catalysis is often accompanied by magnetic type I spin catalysis, induced by spin-orbit coupling [21].

Studies of controlled oxidation by metal ion in liquid phase [1] proceeded simultaneously with the development of mechanisms for enzymatic oxidation [2]. The involvement of iron-dioxygen complexes in a number of enzymatic oxidation processes was first proposed by Warburg around 1920 [7]. Later Hayashi and Mason demonstrated the direct incorporation of O_2 in oxidation reactions catalyzed by phenolase and by pyrocatechase [2]. The first oxygenase model system, the Udenfriend reagent, was reported in 1954, where the mixture of Fe(II) and EDTA, ascorbic acid and O_2 was able to hydroxilate aromatic ring at mild conditions and neutral pH [7]. The ascorbic acid was later replaced by other hydrogen donors and it was realized that in addition to Fe(II) and O_2 all that is needed is a source of electrons and protons [7-11]. The role of spin transformations has not been realized until recent time [3, 18-27].

By spin-catalysis in dioxygen activation one can mean catalysis of hydrocarbon oxidation by the triplet O_2 molecule which involves changes of spin and ion oxidation and does not involve classical free-radical chain autooxidation

mechanism or direct oxidation by a metal salt. The latter obstacle is needed to exclude the Wacker-type oxidation process (2), (3) in which the oxygen in the product is derived from water [2], at the initial step (2) at least:

$$CH_2{=}CH_2 + Pd(II) + H_2O \rightarrow CH_3CH{=}O + Pd + 2H^+ \quad (2)$$

$$Pd + SO_2 + 2H^+ \xrightarrow{Cu(II)} Pd(II) + H_2O \quad (3)$$

It does not matter whether O_2 complex formation precedes or follows the oxidation of the hydrocarbon substrate by an oxometal species [2, 3]. The former pertains to liquid and the latter pertains to gas phase processes. Liquid phase oxidation processes usually involve the free-radical chain mechanism with exceptions of the Wacker process and a few similar ones [1, 2]. In contrast, gas phase oxidations usually involve the mechanism of P. Mars and D.W. Van Krevelen (MVK) [9], which corresponds to direct oxidation of the hydrocarbon substrate by oxometal species, Mo(IV)=O or V(V)=O, followed by regeneration with O_2. The MVK mechanism of substrate (S) oxidation by metal (M) is given by the scheme [9]:

$$\begin{gathered} M(n){=}O + S \rightarrow M(n\text{-}2) + SO; \\ 2M(n\text{-}2) + O2 \rightarrow 2M(n){=}O \end{gathered} \quad (4)$$

Sheldon explains the marked difference between the gas and liquid phase in the folowing way [2]. In liquid phase the facile free radical autoxidation (if it starts somewhere) is ubiquitous in the whole volume of liquid and it is difficult to compete with such radical chain reactions. In the gas phase, on the other hand, the concentrations of hydrocarbon in the vicinity of the catalyst are much lower making radical chain reactions less favorable. The gas-phase oxidation is highly selective but the free-radical autoxidation is usually non-selective (it provides high selectivity only with such substrates as toluene, which contains only one reactive position) [1]. Therefore there is a great need for catalystic methods that are able to compete with free-radical autoxidation in liquid phase oxidation processes, *i.e.* to create gas phase conditions in the liquid phase. Looking at the electronic mechanism of the O_2 activation [3], one can see that the great difference between two types of oxidation processes is determined by the type of overcoming of spin prohibition. In this review we shall see some enzymatic biooxygenation processes which bear a marked resemblance to gas phase oxidations, because they are based on effective overcome of spin prohibition without radical chain conditions.

The role of spin effects in biochemistry has been shown for the first time by L. Poling in 1939 in his study of magnetic susceptibility of iron porphyrins, which easily changed electron spin upon ligands substitution [13]. Ability to change the formal oxidation state of the heme iron ion is easily released in such metabolizing heme proteins like myoglonin, peroxidases, cytochromes [1-3]. The sequence of such electronic pliability is the presence of low-energy states with different spin multiplicity ($m = 2S + 1$) in heme active species, where S is a total spin quantum number; $S^2 = S(S + 1)\hbar^2$ [2]. Iron porphyrins continue to be the subject of active research because they serve as structural models for the active sites of heme proteins. The $3d^6$ Fe(II) ion exhibit three spin states: S = 0 (low-spin, singlet), S = 1 (triplet) and S = 2 (high-spin, quintet); the ground state depends on the field of porphyrin and axial ligand. The iron ion can also be oxidized to an inactive Fe(III), "ferric" form, where it can bind a water molecule, yielding the met-myoglobin or resting form of peroxidases and cytochromes. The resting state of cytochrome P450 with the ferric Fe(III) ion is a doublet (S = 1/2), according to electron spin-echo experiment; the quartet (S = 3/2) and the sextet (S = 5/2) states being very close by energy [4, 5].

For all aerobic organisms on the earth, hemoproteins play a vital role in various life processes. These include the transport and storage of molecular oxygen by hemoglobin and by myoglobin, the transfer of electrons from respiratory substrates by cytochromes and the terminal oxidation with O_2 by cytochrome c oxidase, the decomposition of hydrogen peroxide by catalase, the oxidation of organic substances with H_2O_2 by peroxidase, the hydroxylation of organic substances through dioxygenation by cytochrome P-450. All these different functions involve spin-forbidden nonadiabatic transitions and are based primarily upon the oxidation–reduction properties of the heme iron.

The heme unit itself includes an iron-protoporphyrin-IX active site connected to the globular protein chains by the proximal amino acid residue that serves as an axial ligand to the heme iron. A unique property of the iron-protoporphyrin-IX complex is significant electron and spin delocalization in the Fe-porphyrin system resulting in a reduction of the formal charges ±2 on the both moieties [2]. The pliability of electronic system of the heme macrocycle and its easiness to provide efficient redistribution of atomic charges and unpaired spin density can play a key role in dioxygen binding and in spin-catalysis of heme-containing enzymes [3, 18].

Changes in spin state are important integral properties of the iron enzymes biochemistry. Even the binding of O_2 to the five-coordinated ferrous heme site in myoglobin and hemoglobin (the simplest dioxygen-activation biochemical reaction) is the most striking manifestation of this spin-nonconservation effect [18, 25]. The other striking violation of spin-conservation selection rule is connected with transformation of the doublet (low-spin) ferric resting state of cytochrome P450 to the pentacoordinated ferric porphyrin complex with the sextet (S = 5/2, high-spin) ground state upon substrate binding [6, 20]. This transformation is doubly spin-forbidden, since it includes two-electron excitation and (S = 1/2) → (S = 5/2) transition. Nevertheless it occurs [19, 20] by some mysterious reasons and facilitates the next important

step in the enzymatic hydroxylation cycle, that is one-electron reduction from the ferric to ferrous complex. The reason is well-known now: this is spin-orbit coupling, which induces a stepwise spin transformation through the intermediate quartet (S = 3/2) state [3, 18].

Similar spin-effects are important in catalysis by other metal complexes [36, 37]. Oxidation of cyclohexene in the presence of low-valent complexes of Ir, Rh and Pd affords a mixture of cyclohexen -2-one and cyclohexene oxide [1]. In 1971 it was proposed that the reaction involves an "oxygen activation" mechanism [11]. Nowadays one can say that this is an example of spin catalysis. A number of oxidation processes catalyzed by cobalt Schiff bases, Ni, Pd and Pt peroxo complexes, Co(II)Salen-catalyzed oxygenation of phenols, alcohols and amines [1] constitute models for several oxidations catalyzed by copper-dependent oxygenases [2], for example tryptophan dioxygenase, tyrosinase, copper amine oxygenases [32]. It was concluded that the distinction between mono- and dioxygenases is rather arbitrary and is determined by the fate of the RO_2M intermediate [2]. For the aprotic hydrocarbon substrates, which are unactivated by nucleophilic displacement at the metal, the nature has had to find a different pathway for catalytic oxidation with triplet O_2. A common feature of all of these systems is the intermediacy of a high-valent oxoiron species as the active oxidant [2, 7].

Both nonheme- and heme-dependent oxygenases are known [2-6, 14]. Methane monooxygenase, which mediates the selective methane-methanol conversion is the best example of the former type and illustrates spin-catalysis induced by spin-orbit coupling on the iron ion [34-37]. There are many new examples of similar type of nonheme catalysis [39, 40, 45-47]. The most well-known examples of the latter are already mentioned peroxidases and cytochrome P450 families monooxygenases [4-7]. Spin effects and violations of spin-conservation selection rule are well established now [3-6, 17-20] not only for hemoglobin, but for all these enzymes. [3-6, 17-20].

It is rather strange that the obvious importance of spin-forbidden reactions in the enzymes biochemistry has not received proper attention so far [17-27]. During a spin-state change the enzyme system provides crosses from the potential energy surface (PES) corresponding to one spin state to that of a different spin state; such spin-forbidden biochemical reactions with low-spin ↔ high-spin transitions include changes in the ligand field around the metal center and electron redistribution in the valence 3d-shell of the metal itself. This is a particular requirement, which is necessary to induce internal magnetic field in order to provide a spin flip [3, 18]; that is a relatively strong spin-orbit coupling (SOC) between the two wave functions corresponding to each PES has to be emerged in order to induce avoiding crossing between these two PES. Therefore the question arises: why these spin-transformations are so important for catalytic activation of O_2?

2.1. First Type Spin Catalysis

Spin transitions are also well-known in organic photochemistry. Spins can undergo "depairing" when exposed to light; here an electron goes from doubly occupied orbital $[\uparrow\downarrow]$ of the ground singlet state to a vacant MO $[\ ]$ with the simultaneous spin flip to produce the triplet excited state [14, 15]. According to the Pauli principle, both spins can be parallel (total spin S = 1) in such excited state, when two electrons occupy two different MOs. This triplet state has three possible orientations of the total spin vector, thus the singlet ! triplet excitation includes three possible transitions to three spin sublevels. All of them are spin-forbidden. This is a very strict prohibition, since it can be removed only by influence of magnetic interactions that are much weaker in general than electric Coulomb interactions. The latter determine energetics of chemical bonding, electronic "depairing" excitation and the pathways of chemical reactions. The spin affects the energy through exchange interaction [8]. A weak spin-orbit coupling (SOC) slightly mixes the singlet and triplet states of molecules, which gives a non-zero rate for the T!S transitions that are observed in the form of phosphorescence and are well known as an important quenching processes in photochemistry [14, 15]. The nonradiative T–S transitions also play an important role in the dark reactions, in particular, in catalysis [22, 23]. Weak SOC acts as a "key" needed to open a "heavy door", that is, the system chooses a pathway of chemical reaction with low activation barrier in the triplet state instead of overcoming a high activation barrier in the singlet state. It should be noted that the exchange integral appears with different signs in the energies of the S and T states, namely, two radicals form a chemical bond in the S-state and repel each other in the T-state [10]. The different S- and T-state behavior is important not only for radical reactions, but also for many chemical transformations which include spin "depairing" during bond scission or proceed through biradical intermediate. This often occurs in catalysis by transition metal compounds [2, 22], especially in hemoproteins [3-7].

Flavin enzymes do not contain transition metals, but still activate dioxygen for two-electron reduction and production of peroxides and water. It is assumed [7] that removal of spin prohibition in such reactions proceeds as in the case of radical-chain oxidation, where the spin prohibition can be removed upon formation of primary radicals. It is important to stress a fundamental difference between the enzymatic reactions involving radicals and the radical reactions in chain oxidation processes. In the latter case radicals go to the bulk of the gaseous plasma flame (or in the solution bulk) and do not retain the "spin memory" about precursors any longer. All participants of biochemical oxidation reactions, *i.e.* dioxygen and electron transfer agents, are confined within the same active site

of enzyme. If an electron is transferred to the oxygen molecule from a diamagnetic enzyme M, *i.e.* $O_2 + M \rightarrow O_2^- + M^+$, it produces a triplet radical ion-pair (triplet precursor), all spins remain correlated, the "spin memory" is retained and the spin prohibition to subsequent reactions of the radical ion-pair thus generated is not removed and can not lead to a singlet product.

For example, reaction of O_2 with glucose oxidase (GO) [27] involves flavine adenine dinucleotide (FAD) and includes two half-reactions; namely, glucose oxidation to glucosolactone with reduction of FAD to $FADH_2$ and the reverse cycle $FADH_2 \rightarrow FAD$, with reduction of O_2 to H_2O_2. Plant biomass is a renewable feedstock for chemical industry now. The biomass processing is based on carbohydrates usage, that are transformed into basic organic chemicals by fermentation technologies. Thus glucose oxidation leads to gluconic and glucaric acids, which are very perspective for synthesis of a number of ethers, lactones, *etc.* From the standpoint of dioxygen activation it is interesting to consider only the second half-reaction (Fig. 2). At the fist stage the O_2 molecule comes to the GO active site and occupies a cavity between $FADH_2$ and the nearest protonated histidine residue (Fig. 2a). Then a fast electron transfer occurs, which is almost thermo-neutral according to density functional theory (DFT) calculations [28]. The T→S transition has to occur after formation of a triplet radical pair, $FADH_2 + O_2 \rightarrow FADH_2^{+\bullet} + O_2^{-\bullet}$(Fig. 2b), in order to provide the final products $FAD + H_2O_2$.

The catalytic cycle accompanied by the formation of hydrogen peroxide (Fig. 2c) can occur only in the singlet state. It involves abstraction of hydrogen atom from $FADH_2^+$ and a proton abstraction from the nearest protonated histidine residue in order to create H_2O_2 from the superoxide anion. The final stage (not shown in Fig. 2) includes the proton transfer from N5 atom of $FADH^+$ back to hystidine through the H-bonds network in the peptide chain [11].

The T→S transition has been explained [27, 28] by a relatively large SOC between the S- and T-states of the radical pairs (Fig. 2b), which have different orbital structures inside the superoxide ion. As one can see in the orbital scheme on the top of Fig. 2b, the T- and S-states have different occupations of the $\pi_{g,x}$ and $\pi_{g,y}$ molecular orbitals of the dioxygen moiety; thus the T→S transition includes an electron jump from one $\pi_{g,x}$ molecular orbital of the superoxide ion to another $\pi_{g,y}$ orbital. Such transformation is equivalent to orbital rotation, or to a torque, which creates transient magnetic field; finally this magnetic field induces a spin flip [1, 18]. This simple consideration is supported by direct quantum-mechanical calculations of the SOC integrals [13, 17]. With account of the SOC operator in the effective single-electron form

$$H_{SOC} = \sum_A \zeta_A \sum_i \vec{l}_{i,A} \cdot \vec{s}_i = \sum_i \vec{B}_i \cdot \vec{s}_i = \\ = \sum_i (B_{i,x} s_{i,x} + B_{i,y} s_{i,y} + B_{i,z} s_{i,z})$$

where ζ_A is a SOC constant for atom A (ζ_O = 153 cm^{-1} = = 0.44 kcal/mol), $\vec{l}_{i,A}, \vec{s}_i$ are the orbital and spin angular momentum operators for the *i*-th electron, respectively, one can get the SOC integral for the T-S transition, shown in Fig. 2b, equal to $0.5\,\zeta_O$ = 0.22 kcal/mol. This relatively large magnetic energy can induce a spin flip with a competitive rate constant [28]. That is why the glucose oxidase can produce hydrogen peroxide taking O_2 just from the air. This example of spin catalysis in dioxygen activation, determined by weak internal magnetic perturbation, SOC, can be used as a starting point in developing of new industrial catalysts for environment friendly "no-waste" technologies.

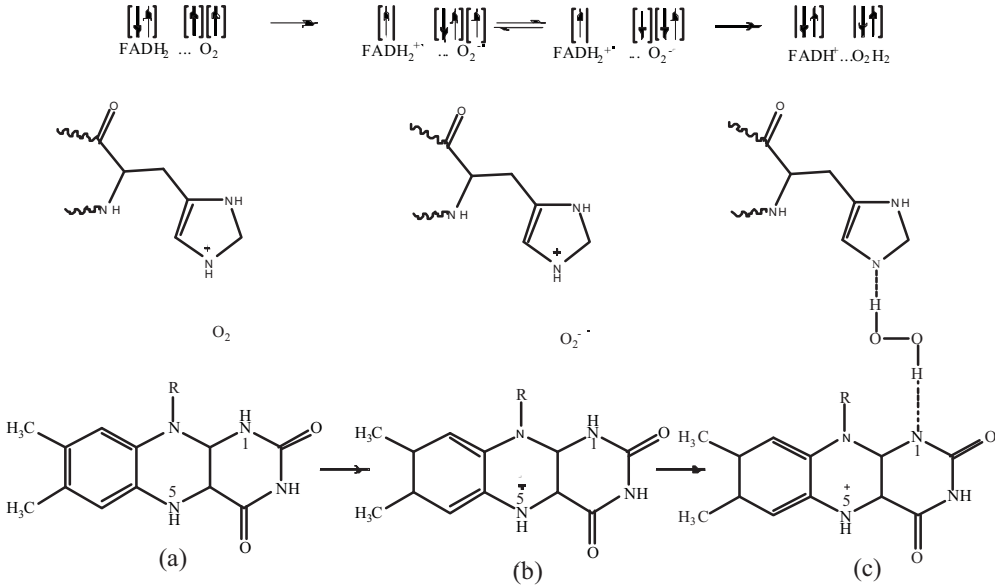


Fig. 2. The most important stages of the O_2 reaction with glucose oxidase

2.2. Alkane Hydroxylation

Alkane hydroxylation by cytochrome P450 is one of the most important processes by which the cells metabolize toxic and endogenous compounds utilizing dioxygen. There are many other iron-containing heme and non-heme enzymes which provide binding and activation of dioxygen in biosystems [1-6]. In general, O_2 binds at the active site and is reduced by two electrons, generating peroxo intermediates. Either one electron is provided by metal or the second by the cofactor, or both electrons are provided by a binuclear metal site [6]. This peroxo species is the first significant oxygen intermediate in many proposed enzymatic mechanisms [2-6]. Hydroxylation of alkanes includes the first step being the cleavage of the C–H bond through H-atom abstraction. The next step of the reaction coordinate corresponds to heterolytic or homolytic O–O bond cleavage [6]. Heme enzymes are able to stabilize a very reactive iron(IV)-oxo species incumbent upon the porphyrin cation-radical.

Epoxidation and hydroxylation of hydrocarbons can be achieved by study and simulation of spin catalysis mechanisms of various enzymatic reactions catalyzed by peroxidases and cytochrome P450 families. Catalytic use of molecular oxygen as a primary oxidant in industrial processes has to find an effective origin in such approach. Since activation of nonpolar inert C–H bond is of great importance for industrial chemistry, the facility with which P450s promote this process makes the cytochrome P450 family a standard masterpiece of Nature for mimetic design of industrial catalysts. Some general mechanisms of enzymatic reactions catalyzed by various hemoproteins are presented in Fig. 3.

In all P450 enzymes a sulfur atom of a proximal cysteine residue is presented as the axial ligand to the heme iron. The cycle begins with the resting state in which a water molecule is bound to the ferric Fe(III) ion (Fig. 3 (1)). The resting state of cytochrome P450 is the most thoroughly studied species; it is a doublet with the ferric ion bearing one unpaired spin (S = 1/2) [19]. The water

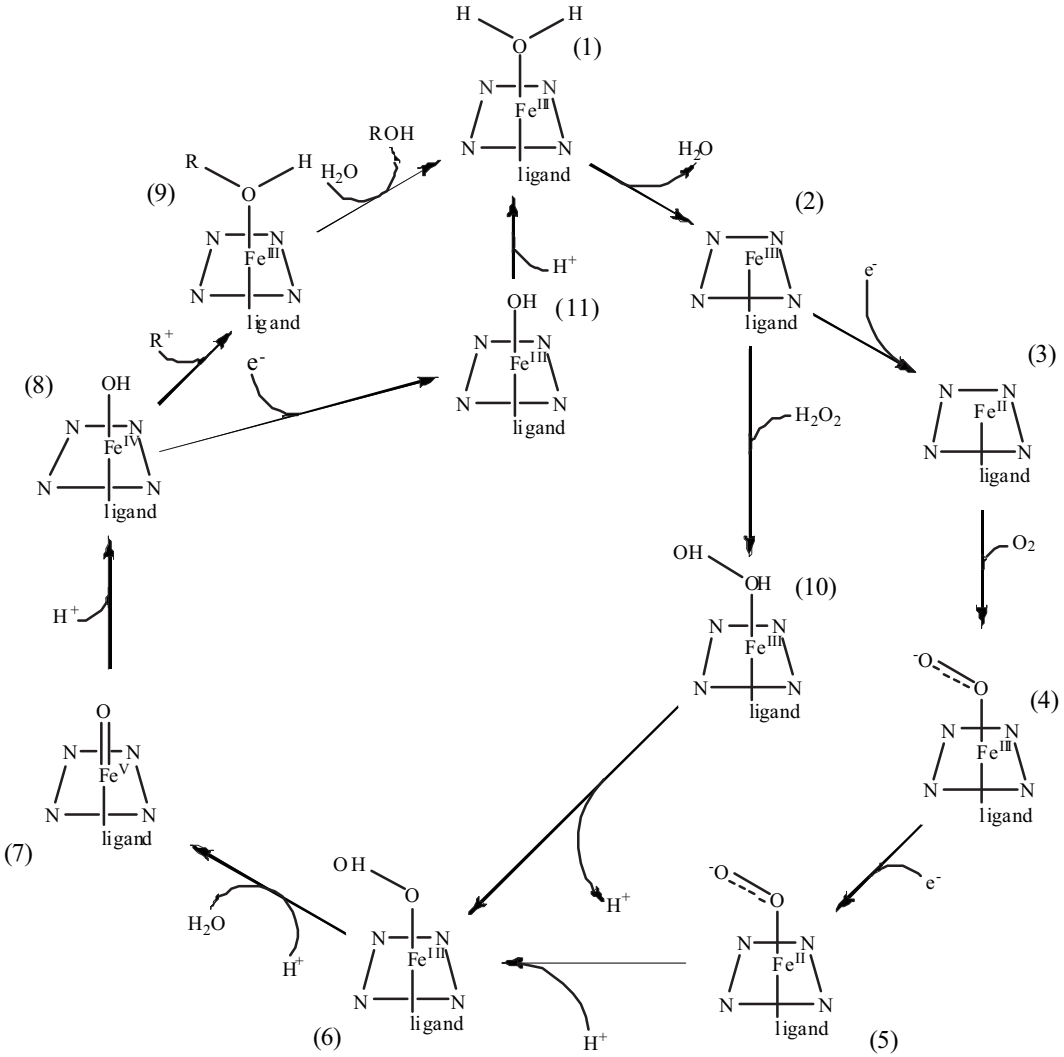


Fig. 3. Catalytic cycle for alkane hydroxylation by cytochrome P450 and by HRP

molecule is an axial ligand separated from the iron by a long distance (2.4 Å) [19]. The spin-catalysis aspects of such enzymatic reactions have not been analyzed so far, though they are of principle importance, especially in terms of biomimetic design of new environmental friendly catalytic processes. In order to discuss spin-catalysis in the enzymatic P450 cycle let us consider spin-orbital configurations for the most important stages (Fig. 4) of these reactions. Usually ferric heme Fe(III) complexes with such a weakly bound sixth axial ligand have a high-spin (S = 5/2) sextet ground state [20], so it was difficult to explain this observation until the DFT calculations were published [19]. In general, the catalytic cycle (Fig. 3) involves sequential changes in the oxidation and spin states, which provide many puzzles from the point of view of ordinary chemistry. Quantum chemistry is an ideal technique to resolve these puzzles and to assess whether the observed properties are consisted with the postulated role of different intermediates in the catalytic cycle (Fig. 3) [19, 20]. In spite of great success of the modern DFT applications [17-25] to variety of different heme and non-heme enzymes, the pure quantum concept of spin-catalysis [34-37] is not well recognized yet. Hydrocarbon RH substrate binding to the distal pocket of enzyme P450 displaces the water and leads to a five-coordinated heme iron (Fig. 3 (2); RH is not shown) in a high-spin (S = 5/2, sextet) state. Thus the doublet-sextet spin transition occurs. This ferric complex is a better electron acceptor and can take up an electron from NADH leading to a high-spin (S = 2) ferrous Fe(II) complex (Fig. 3 (3)). This active site is similar to deoxymyoglobin, thus it can bind O_2 [18]. Such binding yields the oxy-complex (Fig. 3 (4)), which has a singlet spin state of the diradical type [18]. It is an excellent electron acceptor and triggers a second reduction

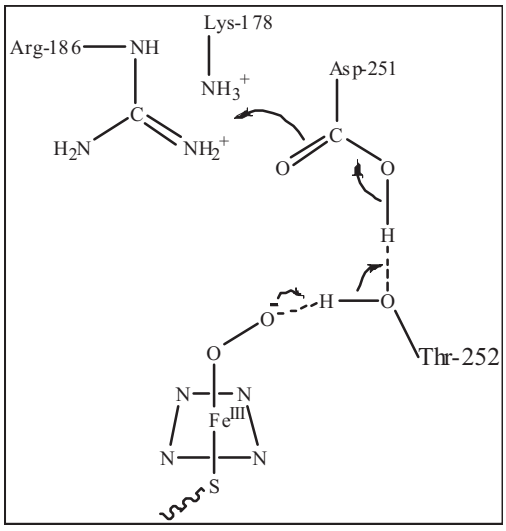


Fig. 4. Elucidation of the (5)→(6) transition (the first protonation step of the terminal oxygen atom) in cytochrome P450, where threonine-252 plays a key role

to produce the peroxo anion (Fig. 3 (5)) [20]. Since this peroxo complex is a good Lewis base, it gets quickly protonated to form the ferric-hydroperoxide species (Fig. 3 (6)), which is called "Compound 0" [19]. The (5)→(6) transition does not include spin flip and all states are doublets. Elucidation of the (5)→(6) transition is given in Fig. 4; this is the first protonation step of the terminal oxygen atom in a proximal region of cytochrome P450, where alcohol-carboxylic acid couple in the vicinity of the active site [19] (threonine-252 and aspargin-251) is important. The threonine-252 residue plays a key role as a primary proton-donor in this protonation step of the terminal oxygen atom in cytochrome P450.

The "Compound 0" is still a good Lewis base and abstracts a new proton to form water and "Compound I" (Fig. 3 (7)). The "Compound I" is illusive, but very important intermediate in a great variety of hemoproteins [1-7, 19, 20]. It was proposed in 1966 [48] and is a commonly accepted species nowadays because of DFT calculations [4] and non direct kinetic evidences [2, 5-8]. In a well-known Udenfriend's reagent, used for the hydroxylation of aromatics [2], a similar oxoiron (V) speices is involved [2, 5]. The "Compound I" transfers an oxygen atom to the hydrocarbon RH substrate, which is converted to an alcohol (Fig. 3 (8→9)). After this catalytic transformation the alcohol exits the active site and a water molecule enters. The enzyme restores the resting state (Fig. 3 (1)) by binding H_2O. All stages of the catalytic cycle have been calculated at different DFT levels with static and dynamic approximations by numerous investigators during the last ten years [4, 5, 19, 20, 39, 43, 54, 55]. Nevertheless, some details of the late stages of the cycle are still debated [4, 39]. The change of a number of electrons at the step 2→3 (Fig. 3) provides a spin transition from (S = 5/2) to (S = 2). Calculation supports that the additional spin has to be paired. Dioxygen binding at the step 3→4 (Fig. 3) provides a spin transition from (S = 2) to (S = 0) [20]. This spin quenching is a very complicated process; it is similar to O_2 binding with such hemoproteins as myoglobin [50-55]. The spin transition was considered in Ref. [18]. The calculated ground state of the reduced ferrous dioxygen heme (Fig. 3 (5)) is a low-spin doublet species, in agreement with EPR data [20]. The O–O bond is shown to be bound to the heme iron in the asymmetric end-on type of structure, similar to that found for Fe(II) porphyrin [54, 55].

Transformation 1→2 (Fig. 3 (4)) includes the substrate binding, which displaces the water in the distal pocket of enzyme P450; it leads to a five-coordinated iron (Fig. 5 (2)) in a high-spin sextet state. Thus this reaction includes spin transition from (S = 1/2) to (S = 5/2) state. Such spin transition ($\Delta S = 2$) is strictly spin-forbidden even if SOC is taken into account [41]. Including SOC perturbation one can permit spin transitions of the type ($\Delta S = 1$), but this permission depends on the value of SOC

integral between two spin states [3]. If the orbital angular momentum is not changed during the transition between these states, the SOC integral is zero and spin transition is still forbidden [34]. The first conclusion from these spin selection rules is that the transformation 1→2 (Fig. 3) can proceed in two steps: transition from (S = 1/2) to (S = 3/2) state and then the next step will include a spin flip from (S = 3/2) to (S = 5/2) state. The first transition corresponds to one-electron excitation $d_{xz} \rightarrow \sigma^*_{x2}$ with a simultaneous spin flip. The corresponding molecular orbitals are strongly localized at the iron ion and the transition includes a great change of the atomic orbital angular momentum. The calculated SOC integral is equal to 92 cm^{-1} and is determined near the crossing of the doublet and quartet sates potential energy surfaces (PES), which occurs along the prolongation of the Fe–O bond length (2.52 Å). The PES crossing is found by DFT method (B3LYP/6-32G) with Gaussian 03 package [49]. The next step transition includes a spin flip from (S = 3/2) to (S = 5/2) state; it corresponds to electron excitation $d_{x2-y2} \rightarrow \sigma^*_{xy}$ with a simultaneous spin flip. This excitation can be induced by a weak change of electric field created by substrate. Since the orbital angular momentum at ferric ion is changed again one can expect a non-zero SOC integral for such spin transition. The resonance Raman, EPR and electronic spectra of HRP, cytochrome c peroxidase and metmyoglobin indicate either a sextet or quartet ground state of their ferric aquo heme complexes [20]. All of them have the histidine residue as a ligand (Fig. 3). By contrast the resting form of P450 (with a cystein ligand) is a low-spin state. The calculated spin-states energy separation in these heme models is really very small. The main conclusion from this study is that stepwise spin transitions are effectively allowed with SOC account.

Harris found [19, 20] strong spin selection in the protonation at the (5)→(6) step; the protonation of the quartet species (Fig. 3 (5)) was shown to be more costly than of the doublet species due to reduced negative charge on the terminal oxygen atom of the high-spin state. The ground state of the ferric-hydroperoxide species (Fig. 3 (6), Compound 0) is doublet with a single electron in the π^*_{yz} orbital (Fig. 4); the quartet state lies 14 kcal/mol higher [20]. Thus the hydrogen-bonding network provides the cytochrome P450 with subtle and spin-selective protonation machinery [19].

The second protonation step (6)→(7) (Fig. 3) leading to Compound I is very fast and involves extremely transient species [19, 20]. The reaction starting from the peroxo anion species (Fig. 3 (5)) and leading to Compound I was found to be exothermic (about 65 kcal/mol) without any reaction barrier and is spin-allowed [4, 19]. During this process the doublet and quartet states are getting close to degeneracy at the stage (7) and spin transformations began to be important during the next hydroxylation reaction. The elusive Compound I was not detected, since it does not accumulate in the catalytic cycle; its existence as a transient species was indirectly supported by cryogenic EPR and visible spectra [20] and also by DFT calculations [4, 6, 19]. The Compound I (Fig. 3 (7)) has three singly occupied MO's: π^*_{xz} and π^*_{yz} orbitals of the Fe=O group, and the a_{2u} MO of the porphyrin moiety (Fig. 5 (7)). The nature of the late MO is fixed for peroxidases (Fig. 6) but for the P450 it appears controversial [19] to depend on the chosen thiolate model and on the sulfur atom interaction with the proximal proteins.

2.3. Biomimetics

Biomimetics are synthetic catalysts whose structure and catalytic features are based on biological enzymatic templates [46]. These catalysts are developed for commercial as well as environmental needs and shown to be very efficient. One of the challenging tasks in biomimetics is the development of analogues of the active sites of the cytochromes P450, which utilize O_2 to catalyze C-H hydroxylation (Fig. 3), C=C epoxidation and sulfoxidation of hydrocarbons [46, 47]. Biochemical treatment of dioxygen reactivity in nature is very instructive in order to mimic it and simulate it for industrial synthesis, since only natural enzymes can make the unique paramagnetic species to be reactive.

The existence of unpaired electrons in stable molecules is very rare indeed [3, 16]. Dioxygen is the only paramagnetic species found in large quantities in the aerobic tissues. It has also low-lying excited states [3].The singlet excited $^1\Delta_g$ oxygen [3, 13] is a metastable species with a relatively short lifetime (few microseconds in water) and long diffusion path [3]. Generation and quenching of the singlet $^1\Delta_g$ oxygen are governed by SOC and other quantum perturbations [3, 41]. But the ground state triplet $^3\Sigma_g$ oxygen bound and transported by hemoglobin is a source of many other quantum effects besides those described above. From a broader perspective, the non-zero electron spin and the concepts of quantum mechanics play a fundamental role for our understanding of the mystery of Life. Spin of dioxygen is a property of the ground state of the molecule and protects O_2 from its involvement into the realm of ordinary chemistry, where the brute force of activation energy just governs all chemical transformations. Dioxygen is protected from brute force *via* the $X^3\Sigma_g$ - $a^1\Delta_g$ energy gap (22 kcal/mol) [3] and *via* spin-prohibition; at the same time O_2 is extremely fragile and easily activated by the presence of a small amount of radicals or by magnetic perturbations [41]. Such quantum spin protection is very important for biological systems, which operate at room temperature and which are extraordinarily complex, diverse, noisy, and "wet". The quantum spin protection of dioxygen reactivity and its dependence on weak internal and external magnetic perturbations is important in connection with the fundamental fact that biological systems are open driven systems.

The complete oxidation of organic materials by dioxygen, to give CO_2 and water, is very exothermic and

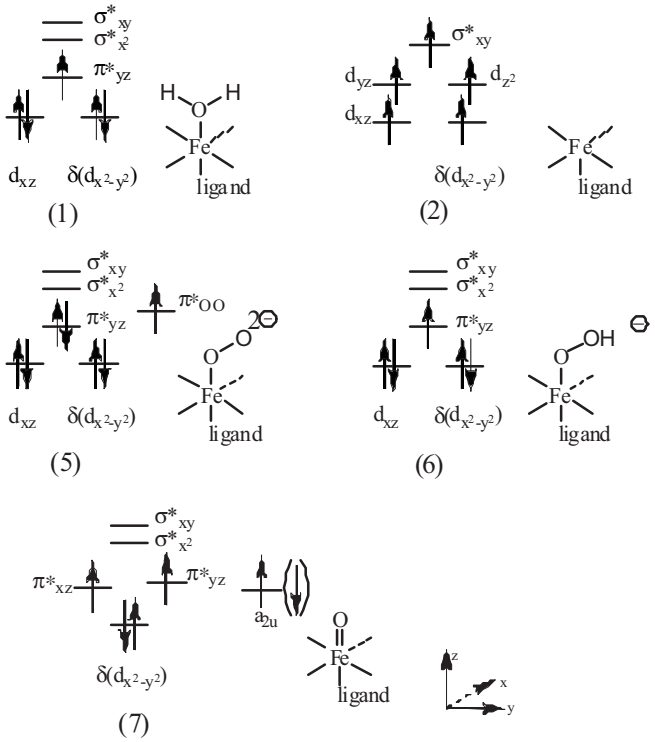


Fig. 5. Spin states of the most important stages. Pentacoordinated ferric- (3) and ferrous-porphyrin (4) have the doublet and quintet ground states, respectively

favorable. Unfavorable kinetic precludes, fortunately, this spontaneous combustion of living matter into "a puff of smoke" [24].

In many heme enzymes, as for example in cytochrome P450, the O–O bond of its low-spin ferric-hydroperoxo intermediate is cleaved heterolytically, generating such Fe(IV)=O(Porhyrin˙)+ intermediate, known as compound I (Comp I). In non-heme iron enzymes such spin-conjugation through d-π system is not available and Fe(V)=O structure is more relevant [6-8]. The first trapped and well-defined oxygen intermediate of this type was that for the bleomycin [7]. This is anticancer drug which provides the DNA strand scission through the H atom abstraction; three different mechanisms of this reaction are proposed [8] and all are important for understanding of the role of electron spin in homolytic and heterolytic O-O bond cleavage [6]. Spin prohibition of O_2 reactions with iron-heme proteins during the catalytic hydroxylation cycles of hydrocarbons by HRP or by cytochrome P450 (Fig. 3) is effectively removed by involvement of paramagnetic Fe ions. But some important preliminary steps (Fig. 3 (1-3)) are determined by SOC in the 3d shell of the iron. These steps correspond to the first type of spin catalysis, though the total hydroxylation cycle (Fig. 3) includes both types of spin catalysis. In this respect the reactions of $^3O_2$ with hemoglobin and myoglobin at moderate temperature are very peculiar, since they are still spin-forbidden [3-5].

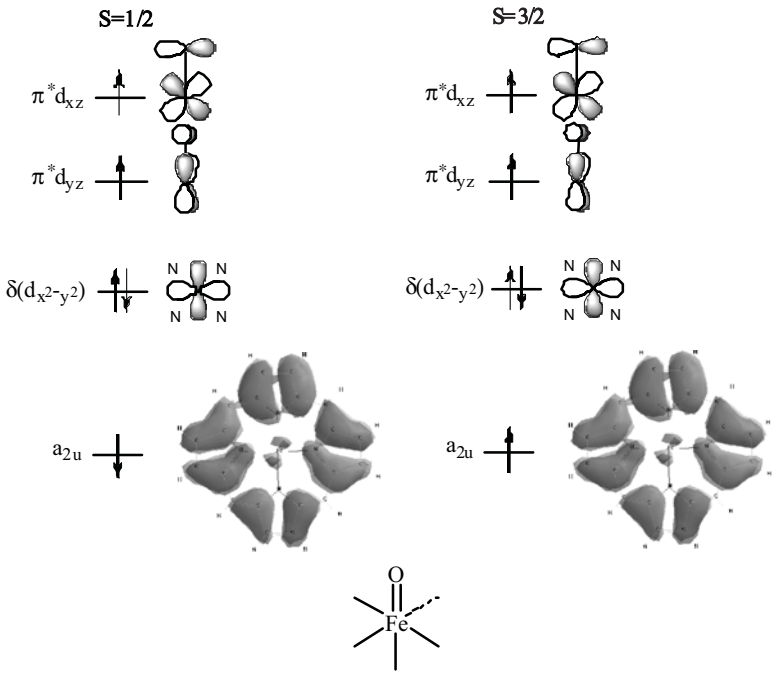


Fig. 6. Spin states of the Compound I

2.3.1. Spin-orbit coupling in the $^3O_2$ reactions with hemoglobin and myoglobin as a model for spin catalysis in dioxygen activation

These most simple biochemical reactions of dioxygen are so effective and important for life that not many people pay attention to their spin prohibition. Hemoglobin and myoglobin are important globular proteins that reversibly bind the O_2 molecule. Myoglobin is found in muscle cells where it stores dioxygen and transports it to the working muscles supplying oxidation energy [3]. Hemoglobin is the O_2 carrier in the red blood cells; it is essentially a tetramer of four myoglobin molecules. Both proteins contain ferrous iron of a heme group, which is usually simulated by Fe(II) porphyrin [25-31], where the iron ion is tetra-coordinated to nitrogen atoms of the tetra-pyrrole rings. The proximal histidine residue from the protein side chain is also bound to the Fe(II) ion leaving one empty position in the octahedral coordination sphere around the ferrous iron. In a simple overview the dioxygen binding to myoglobin can be presented by transition (3)→(4) in Fig. 3. Understanding of dioxygen binding to Fe(II) porphyrin is a key problem in biomimetics and we shall shortly discuss the spin prohibition of this step.

The O_2 binding with myoglobin model has been studied recently by DFT methods [25, 29-31] including account of SOC effects [18]. The optimized potential energy curves (PEC) were calculated for a number of lowest electronic states in Ref. [31], while the PEC for spin states S = 0, 1, 2, 3 at fixed geometry as a functions of the Fe–O_2 distances were presented in Ref. [30]. Recently [18] some points of the fixed Fe–O distance (1.8, 2.0 and 2.5 E) have been recalculated with full geometry optimization of all other parameters for the possible multiplets with account of different symmetries (A' and A") in respect to the O–O–Fe plane. The B3LYP/6-31G* method [49] has been employed and the results quite close to those presented in Ref. [31] have been obtained. All vibrational frequencies and their intensity in the infrared and Raman spectra have been calculated. The Fe-O_2 stretching vibrational frequency is calculated at 539 cm^{-1}, which agrees qualitatively well with the resonance (Soret excitation) Raman band, observed at 567 cm^{-1} for oxy-hemoglonin [51-55]. This indicates applicability of the DFT method and the model used in the work [18].

In the entrance channel of the O_2 binding reaction to heme there is a number of different multiplets. At the infinite separation the deoxyheme has a quintet ground state which is in agreement with the experimental EPR data [51]; there are other indications that the isolated deoxyheme is a high-spin quintet. In this case the porphyrin Fe–N distances (2.08 Å) are larger than in the low-spin states of deoxymyoglobin (2.0 Å) and the iron ion is above the porphyrin ring plane by 0.3 Å in agreement with the x-ray data (0.36 Å) [52]. This illustrates the well-known fact, that the high-spin iron ion Fe(II)(^{5}D) is too large to fit into the porphyrin ring cavity [14]. When this deoxyheme interacts with the triplet ground state dioxygen $O_2(X^3\Sigma_g^-)$, there are six unpaired electrons: four from heme and two from O_2. Their interaction can provide a variety of possible total spin states. The maximum spin corresponds to the septet (^{7}A", S = 3) state, when both sub-systems have parallel spins. If they are antiparallel, the triplet state ^{3}A" occurs. The intermediate quintet ^{5}A" state is also possible. At long Fe–O distances (R > 2.5 Å) all these states are degenerate, because of the absence of exchange interactions between O_2 and heme. The oxygen degenerate π_g orbitals are splitted into the a' and a" symmetry, in respect to the plane. In combinations with 3d orbitals this leads to the A' and A" states, respectively. All spin states which occur at each random collision of heme and O_2 should lead to oxygen binding, but the rate constants would be spin-dependent. It would be different for the triplet, quintet and septet states and even for different spin sublevels of one multiplet. More detailed information about O_2 binding has been obtained in flash-photolysis studies of O_2 dissociation from heme, when the fate of dioxygen depends on the competition between intrinsic recombination rate constant and protein relaxation, as well as the O_2 escape from the protein [6].

The triplet state of deoxyheme, being very close in energy to the ground quintet, produces the adducts with the triplet O_2 which could be either quintet (S = 2), triplet (S = 1), or singlet (S = 0) depending on ferromagnetic or antiferromagnetic coupling of two species. Thus only triplet deoxyheme could provide the ground singlet state product in the process of the antiferromagnetic coupling with dioxygen in a spin-allowed oxyheme formation. Spin transition from the ground quintet to the close lying triplet deoxyheme can be induced by SOC in the 3d shell of iron ion. In this case the primary electronic reorganization takes place in ferrous ion at the equilibrium between the quintet and triplet states already before dioxygen approaches the deoxyheme [18]. All other recombination processes include spin flip induced during heme–O_2 interaction; they seem to be more important for dioxygen binding [18]. Such natural heme–O_2 reactions could start with the ^{3}A"(2) state, which is repulsive at shorter distance (R < 2.5 Å). The optimized singlet ground state ^{1}A'(1) in the reaction product is lower in energy than other multiplets at least by 0.4 eV; this oxyheme is an open-shell singlet [30]. It has a short Fe–O distance (1.81 Å) [30] in contrast to the high-spin states (2–2.7 Å) [17,18,31]. Its structure is close to the Fe^{3+}–O_2^- radical-pair structure [31]. The spin densities in oxyheme are equal to 0.94, -0.31, -0.72 for the Fe–O–O chain, respectively at the optimized bond angle of 118°. The O–O bond distance (1.36 E) and vibration frequency (1110 cm^{-1}) correspond better to superoxide ion [18].

At the intermediate distances 2.5–3 Å the starting ^{3}A"(2) state from the entrance channel transfers to the

triplet Fe^{3+}–O_2^- radical-pair. In this region there are a few crossing points between S- and T-states, including the $^3A''(2)$ –$^1A'(1)$ states crossing, where the spin change could occur [24, 31]. A simplified electronic structure of the $^3A''(2)$ and $^1A'(1)$ states near the crossing of the potential energy surfaces (PES) is presented in scheme (5). The two outer electrons of the ground triplet state of the oxygen molecule in the two degenerate π_g–MO's provide a scheme [↑][↑]; an electron transfer from Fe^{2+} to O_2 produces the radical pair Fe^{3+}–O_2^- (5); it can be accomplished by the occupation of either $\pi_{g,x}$- or $\pi_{g,y}$-orbitals. Both radical pairs could be in the triplet and singlet states; all four states are almost degenerate at the intermediate distances. Now we are interesting only in those spin states which are presented in scheme (5), since only they correspond to the desired T→S transition and to the final product of the O_2 binding to heme. The scheme (5) is equivalent to Fig. 2b and the same explanation can be applied for the high SOC matrix element [18]:

$$\begin{array}{lll} [\uparrow].....[\downarrow\uparrow][\uparrow] \xrightarrow{SOC} & [\uparrow].....[\downarrow][\uparrow\downarrow] & \\ Fe^{3+\bullet}.....O_2^{-\bullet} & Fe^{3+\bullet}.....O_2^{-\bullet} & (5) \\ Triplet, {}^3A''(2) & Singlet, {}^1A'(1) & \end{array}$$

In the triplet entrance channel the starting triplet radical pair Fe^{3+}–O_2^- corresponds to a charge-transfer (CT) state described by the $^3A''(2)$ wave function; this is an electron transfer to the $\pi_{g,x}$-orbital of the O_2 molecule. In scheme (5) the final singlet radical pair corresponds to a CT state described by the $^1A'(1)$ wave function, which presents transfer of an electron to another $\pi_{g,y}$ degenerate orbital of dioxygen. The SOC matrix element between these CT states mounts the maximum possible value (for a system comprised of the light oxygen atoms) [18]. The 3d orbitals at Fe(III) ion also provide an orbital rotation during the triplet-singlet transition and contribute to the SOC integral. The scheme (5) represents only a part of the total wave functions [18] and different occupations of the iron 3d AOs are also possible like in Fig. 5. Since the O-O and O-Fe axes do not coincide (the angle is 118°), the Fe and O_2 contributions to the total SOC integral can not be of opposite sign; they can not quench each other, but only enhance. It is important to stress that the rapid T–S transition in O_2 binding to heme is not only forbidden by spin, but also by orbital symmetry (it includes the A"–A' symmetry change). Such double prohibition is necessary in order to make the spin change in chemical reaction to be effectively allowed [3, 18].

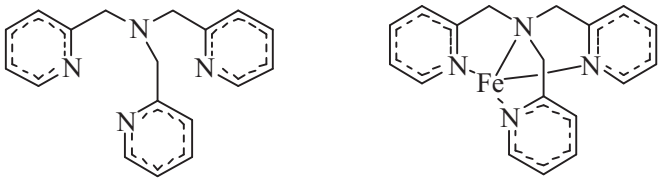
2.3.2. Biomimetic of nonheme iron enzymes which catalyze the activation of dioxygen

Signicant progress has been made in the past 10 years in understanding of how nonheme iron enzymes catalyze the activation of dioxygen in the course of oxidizing a number of substrates [39, 60, 61]. Synthetic chemists have designed suitable simulating complexes that serve as functional models for enzymes, since the modern protein crystallography provides visualization of the enzyme active sites which lead to the activated oxygen species. A lot of high-valent noheme iron-oxo complexes have been synthesized to provide biomimetic oxidations [39, 60-63]. Aliphatic hydroxylation is of particular interest since the oxidative cleavage of such a strong C–H bond is intrinsically difficult [1, 2, 62]. At present there are no direct synthetic methods for specific replacement of unactivated carbon-bound H atom by a hydroxyl group. In the above considered enzymes (Fig. 3) the common mechanism is the subsequent oxidation of iron(II) or iron(III) to the high oxidation state of iron(IV).

In development of nonheme oxygenases a family of iron complexes [Fe(II)(TPA)X_2] was discovered, where TPA is the tetradentate tripodal tris(2-pyridylmethyl)amine ligand (Fig. 7) in the following denoted by L, X is typically acetonitril ligand [60, 61]. These complexes demonstrate interesting catalytic activity and utilize hydrogen peroxide to carry out highly stereoselective alkane hydroxylation and olefin epoxidation. The high stereoselectivity of the oxidation excludes an involvement of OH radical and imply instead a more selective oxidant such as Fe(III)–OOH or Fe(V)=O intermediates [61]. DFT calculation of the reaction mechanism indicates that a high-valent iron-oxo intermediate is formed, where Fe(V) oxidation state is attained [60]. In contrast to the analogous Compound I in cytocrome P450 or in HRP, where porphyrinis is ionized (Fig. 6), the TPA ligand is not oxidized and electrons are extracted from iron center providing the high oxidation state Fe(V). Before the catalytic alkane oxidation step, the Fe(III)(TPA)-OOH intermediate is generated from the reaction of Fe(II)(TPA) complex with the hydrogen peroxide. Solvent water then binds to the Fe(III)(TPA)-OOH intermediate to promote the heterolytic cleavage of the O–O bond (6) by formation of new intermediate (6a), which consequently losses water and generates highly reactive intermediate (6b). The late species (6b) is responsible for the stereoselective alkane hydroxylation [60, 61].

$$\begin{array}{ccc} LFe(III)—OOH & \longrightarrow & LFe(V)=O + H_2O \\ | & & | \\ HOH & & OH \\ a & & b \end{array} \qquad (6)$$

A low energy pathway for the conversion (6) includes a spin transition from the doublet state species (6a) to the quartet ground state species (6b). DFT calculation shows [60] that the complex with water (6a) has one nonpaired electron in the ground state, while the quartet state is higher in energy by 33 kJ/mol. At the doublet state potential energy surface (Fig. 8) after the transition state for the O–O bond heterolytic cleavage there is a shallow minimum, which corresponds to a weak complex



TPA Fe(II)(TPA)

a b

Fig. 7. TPA ligand and its complex with iron ion

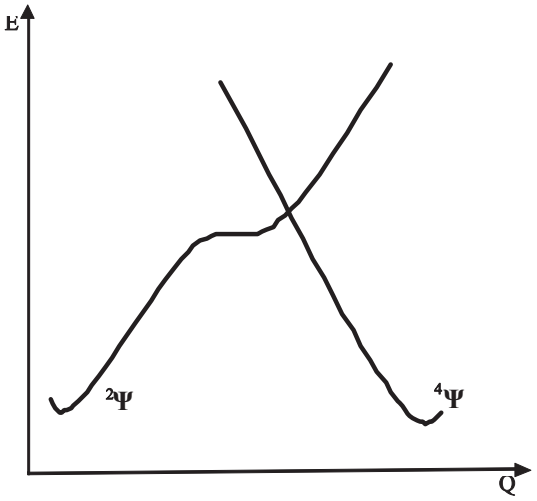


Fig. 8. The doublet and quartet potential energy curves for the heterolytic O–O bond cleavage catalyzed by the Fe(III)(TPA)–OOH species: reaction (6a)→(6b) is spin forbidden

between the new species (6b) and a water molecule. Almost cleaved O–O distance in this complex is about 2.05 Å [60], like in the transition state. A similar minimum is found in the lowest excited state of the hydrogen peroxide [60]. Thus to complete the O–O cleavage, still another electron has to be transferred into the σ*(O–O) orbital, which is supplied by the iron. The unpaired electron left on the iron has to be ferromagnetically coupled to other two nonpaired electrons (Fig. 5 (7)) of the arising Fe=O bond to reach energetically favorable iron-oxo compound according to the Hund rule. Thus a doublet-quartet spin-crossing is needed together with the above mentioned electron transfer (Fig. 8). Spin-orbit coupling at the spin-crossing region is relatively very high, since it includes large contributions from oxygen and iron atoms. It should be noted that the considered picture of the O-O cleavage has large similarity to the one previously found in cytochrome oxidase [29, 60]. Thus the oxidation by non-heme iron complex [Fe(II)(TPA)X_2] has an important common feature of spin catalysis, which are typical of heme peroxidases, cytochrome P450 and other monooxigenases.

2.3.3. Biomimetic comparison with inorganic oxidants

A well-known Fenton's reagent, a mixture of hydrogen peroxide and ferrous salts, provides an extensive chemistry [1, 2] used in industrial applications (for instance, paper bleaching and cleaning of polluted soil); this is very useful as a precedent and guidance in analysis of enzyme reactions [62]. According to the generally accepted Haber-Weiss mechanism [64], oxidation of organic molecules by the Fenton's reagent occurs *via* initial one-electron reduction and iron-catalized dissociation of hydrogen peroxide to yield a free hydroxyl radical with subsequent H abstraction reaction. An alternative explanation, which was first proposed by Bray and Gorin seventy years ago [64], includes formation of a high-valent iron oxo species, the ferryl ion intermediate, Fe(IV)=O, in such iron-peroxide (non-enzymatic) systems. Competition of OH radical and ferryl ion pathways for the Fenton's reagent mechanism has been discussed in terms of equilibrium between Fe(III) + OH and Fe(IV)=O intermediates [62]. An extremely short lifetime of both proposed highly reactive intermediates makes the direct experimental verification rather difficult. The elucidation of the mechanism is further complicated by dependence on reaction conditions (ligands at iron ion, organic substarte, solvent, pH, *etc.*)

Oxidation of cyclohexanol by the ferrous perchlorate and hydrogen peroxide mixture in acetonitrile provides cyclohexanediols in addition to cyclohexanone and observed stereoselectivities supports the formation of a ferryl-ion intermediate and this could not be explained by a freely diffusing hydroxyl radical [62]. Two aspects of enzymatic hydroxylation that are striking in comparison to powerful Fenton's oxidants are the following: (i) oxygen transfer without equilibration with water, and (ii) oxidation with net retention of configuration at the functionalized carbon [62].

On the basis of pH-dependence of the Fenton's reagent chemistry it was concluded recently [66] that a ferryl species but not the OH radical is produced by aqueous Fe(II) and hydrogen peroxide mixture. The results were interpreted in terms of prototropic equilibrium between Fe(III) + OH and Fe(IV)=O ions [62, 66]. The recent DFT study of the Fenton's reagent mechanism [67] supports the conclusion that formation of the ferryl ion from Fe(II)–OOH precursor is very easy in accordance with DFT studies of cytochrome P450 and similar peroxides [4, 6, 39, 61-63]. Thus comparative studies of enzymatic oxidation and the Fenton's reagent mechanism provide useful interpretation of these powerful chemical oxidants.

2.4. Second Type of Spin Catalysis in Dioxygen Activation with Binuclear Metal Centers

The second type of catalytic overcoming of spin prohibition is more relevant to industrial processes, which involve dioxygen for organic feedstock oxidation.

In the context of dioxygen activation we have to mention enzymes with binuclear metal centers, like cytochrome oxydase [12, 22], hemerytrin and hemocyanin [50, 51]. Generally speaking, the enzymes containing iron and copper active sites [18, 50] play key roles in dioxygen activation by generation of a peroxo intermediate; either O_2 is reduced by two electrons, provided by a binuclear metal site, or one electron is provided by metal and the second electron by a cofactor [18].

Cytochrome oxidase catalyses the four-electron reduction of oxygen molecule to water [7]. No intermediates were detected in the reaction $O_2 + 4e^- + 4H^+ = 2H_2O$. However, many experimental measurements [7] have proved the formation of O_2^{2-}. The reaction centre of cytochrome oxidase includes one heme ferrous ion and one copper ion. The oxygen molecule binds to the heme Fe^{2+} cation and to Cu^+ ion that donate one electron each to form an O_2^{2-} anion. This provides a way to overcome the major obstacle to oxygen activation, that is, spin inversion (T–S transition). The O_2^- and O_2^{2-} species have the following ground state configurations: O_2^- ($^2\Pi_g$) and O_2^{2-} ($^1\Sigma_g^+$), respectively. Since the O_2^{2-} dianion has a filled electron shell, the ground state of this species is totally symmetrical. Transfer of two electrons causes the ground-state term, $^3\Sigma_g^-$ of the O_2 molecule, to transform smoothly to the term $^1\Sigma_g^+$ of the dianion. The spin-orbit coupling between the states $^3\Sigma_g^-$ and $^1\Sigma_g^+$ is symmetry-allowed [3]; therefore, the reduction $O_2 \rightarrow O_2^{2-}$ is also symmetry allowed with inclusion of SOC. Transition of the active site of cytochrome oxidase to the singlet state removes spin prohibition for subsequent fast chemical reactions up to formation of stable diamagnetic products [3]. The orbital doubly degenerate ($^2\Pi_g$) ground state of the O_2^- ion is split by strong SOC [3], since it has a nonzero orbital angular momentum. This is an important key aspect of many enzymatic O_2-activation reactions, considered in the previous sections.

3. Conclusions

Selective catalytic oxidation of organic molecules has been a longstanding goal in chemical research and continues as an important reaction pathway for the synthesis of primary and special chemicals. Catalytic utilization of dioxygen with soluble metal complexes in homogeneous catalysis is very important today, and will become even more important in the near future since the worldwide environmental policy becomes more rigid and severe [69]. Development of new and improved processes using dioxygen is driven both by its low cost and by its potential to be environmentally friendly and be a better oxidant than other traditional inorganic oxidants such as chlorine. Nature in its need for oxidative metabolism with carbohydrates and other nutrients has developed the dioxygen-activation catalysts (oxidases, HRP, cytochrome P450) based on iron-heme proteins [1-6]. We can see that spin-dependent quantum effects and transformations are important in a number of such biochemical processes connected with dioxygen activation [3-6, 17-47]. Spin is a fundamental quantum phenomemnon associated with the structure of space-time [3]. The occurrence of non-zero electron spin in biological system indicates that we are in the domain of the life science, where classical biochemical concepts are insufficient for its proper description and understanding.

The complete oxidation of organic materials by dioxygen, to give CO_2 and water, is very exothermic and favorable. Unfavorable kinetic precludes, fortunately, this spontaneous combustion of living matter into "a puff of smoke" [2]. Thus, the direct reaction of triplet dioxygen with singlet organic molecules is a spin-forbidden process with a very low rate, since it can not give stable diamagnetic products in a single elementary step without the spin flip. The usual way of circumventing this kinetic barrier *via* free radical pathway (combustion) is impossible for living cells. The reaction of singlet (diamagnetic) molecule with $^3O_2$ forming two radicals is a spin-allowed process. Usually it is a highly endothermic process; Sheldon provides as an example of such reaction the activation of $^3O_2$ by flavin-dependent oxygenases at moderate temperature [2]. He argues that it is possible only for very reactive substrates (flavins) that form resonance stabilized free radicals, *e.g.* reduced flavins, like $FADH^{\cdot}$, and HO_2 as a counterpart. It is shown in this review that such arguments are non reliable and spin catalysis should be considered. We have stressed, that even in this case the complete oxidation in the enzyme (*e.g.* glucosoxidase) is spin-forbidden, if the HO_2 radical is not the final product, which leaves the active center of enzyme and goes to the bulk of the cell. We have shown that flavin-dependent oxygenases provide a very efficient way to overcome the spin-prohibition by spin-orbit coupling (SOC) perturbation at the stage of electron transfer (Fig. 2b).

The second way to overcome the spin conservation obstacle is for $^3O_2$ to combine with a paramagnetic transition metal ion [21, 27]. Such reactions are spin-allowed and governed by exchange interactions (exchange-induced spin-catalysis) [21]. Peroxo-, dioxo- and superoxo-complexes with metals in different oxidation degree are considered during the search of "dream reactions" for selective catalysts [2, 7, 24]. The expectation that the resulting metal-dioxygen complexes may react selectively with organic substrates at moderate temperature forms a background for the extensive studies of metal-catalyzed oxidation during the last four decades [1-12]. In this respect the reactions of $^3O_2$ with hemoglobin and myoglobin at moderate temperature are very peculiar, since they are still spin-forbidden [18, 25, 29-31].

SOC between the starting triplet state in the O_2 binding to glucose oxidase, to heme, and in a number of

high-spin low-spin multiplet transformation in peroxidases are considered. Both triplet (T) and singlet (S) states are dominated by the charge-transfer structures. Account of specific SOC in the open π_g-shell of dioxygen permits to explain the T–S transitions probability in the active site near the transition state. The SOC model explains well the efficient spin inversion during the O_2 binding with heme and glucose oxidase, which constitute a key mechanism for understanding of dioxygen activation.

Acknowledgments

This work is supported by the State Foundation of Fundamental Investigations (DFFD) of Ukraine, the project F25.5/008.

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СПРИЯТЛИВИЙ ДО ОТОЧУЮЧОГО СЕРЕДОВИЩА СПІН-КАТАЛІЗ АКТИВАЦІЇ КИСНЮ

Анотація. *Каталіз контрольованого селективного окиснення вуглеводнів з використанням молекулярного кисню, будучи дуже важливим для екологічно чистої хімічної промисловості, повинен бути розроблений по аналогії з біологічними ензиматичними реакціями. Каталіз окиснення вуглеводнів парамагнітним киснем, що уникає класичний механізм вільно-радикальних ланцюгових реакцій, потребує подолання заборони за спіном. У цьому огляді представлена класифікація спін-каталізу в біологічній активації кисню, яка обговорена у зв'язку з відомими промисловими процесами. Декілька важливих прикладів подано для ілюстрації ролі спінових ефектів у зв'язуванні* O_2 *міоглобіном, глюкозооксидазою, цитохромом P450, пероксидазою з коренів хрону та негемовими комплексами заліза.*

Ключові слова*: ферум(III) алкілпероксокомплекси, гемоглобін, міоглобін, глюкозооксидаза, цитохром P450, Fe(IV)-оксосполука I, спін-орбітальна взаємодія, спінові переходи.*