Spin-dependent binding of dioxygen to heme and charge-transfer mechanism of spin-orbit coupling enhancement

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Summary. Spin-orbit coupling (SOC) between the starting triplet $^3A''(2)$ state from the entrance channel of the heme-O_2 binding reaction and the final singlet $^1A'(1)$ open-shell state, which are dominated by the Fe^{3+}-O_2^- radical-pair structures, is studied. Simulated potential energy surface cross sections along the reaction coordinate for these and other multiplets, calculated by density functional theory (DFT) agree with the recent DFT studies known from the literature. The heme-model includes Fe(II)-porphyrin complex with imidazol, or ammonia molecule, at the fith coordination position, which simulates the hystidine as an aminoacide residue of myoglobin. The SOC is induced mainly at the oxygen moiety by an orbital angular momentum change in the π_g-shell during the triplet-singlet transition. This SOC model explains pretty well the efficient spin inversion during the heme-O_2 binding.

Keywords: hemoglobin, myoglobin, cytochrome oxidase, spin-orbit coupling, triplet-singlet spin inversion, radical-pair structures, charge-transfer states, binding of diatomic ligands to heme.

Introduction. 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 provides it to the working muscles supplying oxidation energy [1]. 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, where iron ion is tetra-coordinatied to nitrogen atoms of the tetra-pyrrole rings [1-4]. 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. Hemoglobin and myoglobin bind several small gaseous molecules besides dioxygen, e.g. CO and NO [1-3].

The binding of these diatomic ligands to heme has been studied in biochemistry for over

hundred years [2]. It is well-established that the structure of surrounding protein affects the ligand binding ability of the heme group; carbon monoxide binds to free heme in solution much stronger ($2 \cdot 10^4$ times) than dioxygen, but in myoglobin this factor is reduced to 25 times [4]. Thus, myoglobin seems to favour the O_2 binding before CO by 17 kJ/mol [1, 2, 4]. Such discrimination is of vital importance: without it we would suffocate from CO that is produced in our body during metabolism. The reason of the discrimination was connected with different geometric parameters of O_2 and CO binding to the hem iron: the Fe-C-O bond is linear, whereas Fe-O-O bond is bent [4]. Hemoglobin and myoglobin have a second, so-called distal, histidine ligand (His-64) positioned above the Fe(II) ion, but too far to coordinate directly to the iron. His-64 is in the right position to affect the Fe-CO group and to produce a tension. This idea was supported by early crystal structures, showing Fe-C-O angle of 120-130° [4], thus the idea penetrates into the textbooks [4, 5]. Some newer x-ray measurements, IR spectra and DFT calcula-

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tions indicate that the Fe-C-O angle is nearly linear in heme models; thus the idea of a strongly bent Fe-C-O unit as the reason of the discrimination is nowadays agreed to be incorrect [1, 3]. Recent DFT calculations show that the FeO_2 group is much more polar than the FeCO group in hem models [1, 3, 6]. The electronic structure of FeP complex with dioxygen is close to that of a superoxide anion bound to a ferric ion and this charge-transfer complex is in the singlet spin state [1-4]. Thus electrostatic interaction between distal histidine and $Fe(III)-O-O^-$ group is stronger than that for the Fe-CO group; therefore the protein discriminates between O_2 and CO by hydrogen bonding and electrostatic interaction in myoglobin [1-3].

Another important factor of such discrimination is determined by electron spin [2, 3, 6]. Spin is a general intrinsic quantum property of electron. Spin is an angular momentum with a length of the momentum vector $\sqrt{\vec{S}^2} = \sqrt{S(S+1)}\hbar = (\sqrt{3}/2)\hbar$, where spin quantum number for one electron is equal to S=1/2 and two projection $M_s = \pm(1/2)\hbar$ described by α and β wave functions exist (the doublet state) [6]. The role of electron spin in chemistry is well-recognized in terms of spin-valence concept: a covalent chemical bond is formed when atomic orbitals of two electrons with opposite spins ($\alpha\beta$-$\beta\alpha$) overlap each over. This spin wave function is antisymmetric in respect to permutation of two electrons. The total spin of such pair is zero (S=0) and the state is singlet (the only one state; no spin, no intrinsic magnetic moment, the molecule is diamagnetic). When spins are parallel ($\alpha\alpha$, $\beta\beta$ and $\alpha\beta+\beta\alpha$) the total spin has a quantum number S=1 in the equation for the length of the total spin angular momentum

$$\sqrt{\vec{S}^2} = \sqrt{S(S+1)}\hbar = \sqrt{2}\hbar \quad (1).$$

and there are three spin states with different projections on z-axis $M_s = \pm\hbar, 0$ (triplet state). For a simple covalent σ-bond (like in H_2 molecule) the triplet sate is unstable and molecule can exist only in the singlet state with two opposite spins. That is why almost all stable organic molecules contain an even number of electrons, which can be divided into α and β pairs. They are said to have paired spins or to be in the singlet ground state and are diamagnetic.

Such diamagnetic substances normally do not change zero spin during chemical reactions [6-10]. An absence of the total electronic spin in stable molecules leads to illusion that spin is not important in organic chemistry and biochemistry. In fact, the total electronic spin is the main regulating factor in many metabolic processes catalyzed by metal-organic enzymes, such as cytochromes, horseradish peroxidase, copper-aminoxidase [6-24]. Spin inversion is especially important for many dioxygen reactions, binding to hem, combustion and respiration [10]. The importance of spin inversion is also reflected in the Perutz model of the hemoglobin cooperativety [2-4].

Dioxygen molecule is a famous exception to the general rule: unlike many chemically stable organic compounds the O_2 molecule has the triplet ground state, Eq. (1). According to Hund's 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 is paramagnetic (it has intrinsic magnetic moment due to spins of two unpaired electrons) and its 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) [10]. This is the reason why organic matter may exist in the oxygen-rich atmosphere. Because of the spin prohibition, combustion of organic fuels requires activation in the form of high-temperature ignition stage [10], i.e., generation of primary radicals. Reaction of $R^.$ radical (one unpaired electron, S=1/2, doublet state) with O_2 molecule is spin-allowed, since the starting reactants ($O_2+R^.$) and product ($RO_2^.$) both have the doublet states, which provide the radical chain character of the combustion reactions [10]:

$$[\![\uparrow]\!][\uparrow]\!] + [\downarrow] \Rightarrow [\![\uparrow]\!][\downarrow]\![\uparrow]\!]$$
$$(O_2 \; + \; R\cdot) \quad\quad RO_2\cdot \quad\quad (2).$$
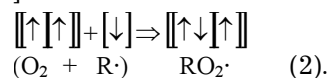
Here the quantum cell [↑] denotes molecular orbital (MO) with α spin. The radical $RO_2^.$ can decompose into radical $RO^.$ and biradical $^.O^.$ thus providing branching chain reaction. In radical chain combustion the energy is released in the form of heat and light without any specific control (until the fuel exhaustion). Clearly, 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 the strictly controlled energy of oxidation of organic compounds in their reactions with dioxygen, overcoming spin prohibition without high-temperature ignition step of radical chain [18]. 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 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 [18].

The O_2 molecule in the triplet ground state has the following electronic configuration $(1\sigma_g)^2$ $(1\sigma_u)^2$ $(2\sigma_g)^2$ $(2\sigma_u)^2$ $(3\sigma_g)^2$ $(1\pi_u)^4$ $(1\pi_g)^2$. The two outer electrons in two degenerate $1\pi_g$-MO's 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-MOs 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, the formation of the latter being strongly dependent on the presence of electron donors (enzymes) and magnetic perturbations that affect the spin prohibitions.

In this paper we want to understand the spin-dependent mechanism of the O_2 binding step once dioxygen has moved from the solvent through the protein α-helices and reached the myoglobin distal cavity near the ferrous-heme cofactor active site. This binding step which has been extensively studied after flash-photolysis [2-4] shows interesting kinetic features, like non

homogeneous decay, recombination barriers etc., and indicates complicated spin-dependence (especially in comparison with NO and CO binding to myoglobin). Explanation of such spin-dependence is the main purpose of this work.

Spin-dependence of dioxygen reactions. Spins can undergo «depairing» when exposed to light; here an electron goes from doubly occupied orbital [↑↓] to a vacant MO [ ] with the simultaneous spin flip [12, 13]:

$$\|{\uparrow\downarrow}\| \|]+ h\nu \Rightarrow \|{\uparrow}\|{\uparrow}\| \quad (3).$$
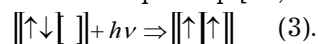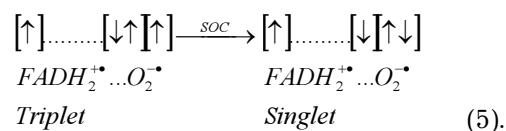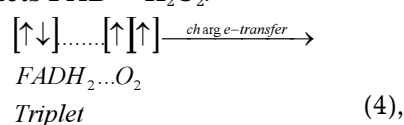
According to the Pauli principle, both spins can be parallel (total spin S=1) in the excited state. This triplet state has three possible orientations of the total spin vector, thus the singlet → triplet excitation includes three possible transitions to three spin sublevels. All of them are spin-forbidden. This is a very strict prohibition, since it can be removed only by influence of magnetic interactions that are much weaker 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. A weak 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 important quenching processes in photochemistry [12, 13]. The nonradiative T-S transitions also play an important role in the dark reactions, in particular, in catalysis [10]. 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. Remind 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 [8], especially in hemoproteins [1-7].

Cytochrome oxidase catalyses the four-elec-

tron reduction of oxygen molecule to water [5]. No intermediates were detected in the reaction $O_2+4e^-+4H^+=2H_2O$. However, many experimental measurements [5] 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). Since the O_2^{2-} dianion has a filled electron shell, the ground state of this species is totally symmetrical and characterized by the term $^1\Sigma_g^+$. 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 [10]; 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 [10].

It is often assumed that one can overcome the spin prohibition to oxidation of organic substrates with atmospheric oxygen by successive addition of single electron and proton in the successive reduction of O_2^-. Further reactions of diamagnetic hydrogen peroxide, produced in such reduction, are spin allowed. It is assumed [5] 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 gasous plasma flame (or in the solution bulk) and do not longer retain the «spin memory» about precursors. 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 O_2 with glucose oxidase [17, 24] involves flavine adenine dinucleotide (FAD) and includes two stages; 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. From the standpoint of dioxygen activation it is interesting to consider only the second stage. After formation of a triplet radical pair, $FADH_2 + O_2 \rightarrow FADH_2^{+\bullet} + O_2^{-\bullet}$, the T$\rightarrow$S transition has to occur in order to provide the final products $FAD + H_2O_2$.

$$\left[\uparrow\downarrow\right]......\left[\uparrow\right]\left[\uparrow\right] \xrightarrow{charge-transfer}$$
$$FADH_2...O_2$$
$$Triplet \qquad\qquad (4),$$

$$\left[\uparrow\right]........\left[\downarrow\uparrow\right]\left[\uparrow\right] \xrightarrow{SOC} \left[\uparrow\right]........\left[\downarrow\right]\left[\uparrow\downarrow\right]$$
$$FADH_2^{+\bullet}...O_2^{-\bullet} \qquad\qquad FADH_2^{+\bullet}...O_2^{-\bullet}$$
$$Triplet \qquad\qquad Singlet \qquad (5).$$

The last phase of the catalytic cycle accompanied by the formation of hydrogen peroxide can occur only in the singlet state. It involves abstraction of hydrogen atom from $FADH_2^+$ and a proton from the nearest histidine residue with subsequent proton transfer to histidine across the system of H-bonds in the protein chain [10]. The T$\rightarrow$S transition has been explained [10] by a relatively large SOC between the S and T states of the radical pairs (5), which have different orbital structures inside the superoxide ion. As one can see in scheme (5), the T$\rightarrow$S transition includes an electron jump from one $\pi_{g,x}$ molecular orbital of the dioxygen 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 [24]. This simple consideration is supported by direct quantum-mechanical calculations of the SOC integrals [10, 15, 17]. In the following we want to show that a similar mechanism of SOC enhancement by charge transfer can be applied for spin-dependent reaction of dioxygen binding to heme.

Materials and methods. The O_2 binding with myoglobin model was studied recently by DFT methods [2, 3, 8, 9]. Fully relaxed potential energy curves (PEC) were calculated for the seven
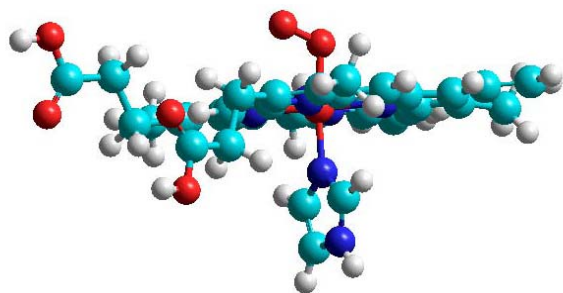
Fig. 1. Ferrous ion in protoporphyrin IX coordinated with O_2 and with imidazol as a proximal histidine residue. This model of heme-cofactor has the singlet open-shell ground state which is close to more symmetric Fe(II)-porphine-imidazole-O_2 model.

Fig. 2. Fe(II)-porphine-NH_3-O_2 model also used in this work for DFT calculations.

lowest electronic states in Ref. [3], 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. [2]. In this work we have recalculated some points R(Fe-O)=1.8, 2, 2.5 Å with full geometry optimization of other parameters for all possible multiplets and accounting for different symmetries (A' and A") for the singlet and triplet states. The model of oxyheme is shown in Fig. 1, which includes protoporphyrin IX coordinated with imidazol as a proximal histidine residue. Its calculation gives the same singlet ground state as for the simplified Fe(II)-Porphin-Imidazole-O_2-model, which poses C_s symmetry [3]. More simple Fe(II)-Porphine-NH_3-O_2 model (Fig. 2) has also been used in this work for DFT calculations in the vicinity of the equilibrium. The B3LYP/6-31G* method [11] has been imployed and the results quite close to those presented in Ref. [3] have been obtained for the Fe(II)-Porphin-Imidazole-O_2-model. For the model, shown in Fig. 2, all vibrational frequencies and their intensity in the infrared and Raman spectra have been calculated. Many normal modes are similar to those, calculated in Refs. [6, 25]. An additional Fe-O_2 stretching vibrational frequency is calculated at 539 cm^{-1}, which agrees qualitatively well with the resonance Raman band, observed at 567 cm^{-1} for oxy-hemoglobin by Soret excitation [20]. This indicates reliability of the chosen model and of the DFT method used in this work.

Results and discussion. At the infinite separation the deoxyheme has a quintet ground state with the triplet state being very close in energy. This is in agreement with experimental data,
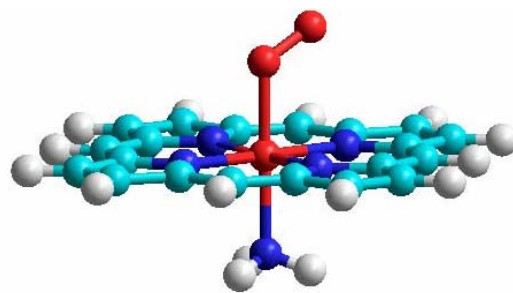
showing that the isolated deoxyheme is a high-spin quintet [1-9]. The optimized structure of this Fe(II)P complex with imidazol at the fifth coordination position agrees with the x-ray analysis of the crystal structure of deoxymyoglobin: Fe-N distances (2.08 Å) in FeP are larger than in the low-spin states (2.0 Å) and the iron ion is above the porphyrin ring plane by 0.28 Å in agreement with the x-ray data (0.36 Å) [2, 3]. This illustrates the known fact, that the high-spin iron ion is too large to fit into the porphyrin ring cavity. When this deoxyheme interacts with the triplet ground state dioxygen, there are six unpaired electrons. Their interaction can provides the septet ([7]A", S=5) state, when both subsystems have parallel spins. If they are anti-parallel, the triplet state [3]A" occurs. At long Fe-O distances (R>2.5 Å) these states are degenerate (together with the intermediate quintet [5]A" state). The A" symmetry is determined by the oxygen degenerate π_g orbitals, which have a' and a" symmetry in respect to the plane, which contains O_2 and imidazol molecules being coplanar during the reaction. In general all spin states which occur at each random collision of heme and O_2 should lead to oxigen binding, but with different rate (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 [2, 7]. The great different recombination dynamics of O_2, CO and NO molecules with heme have been attributed to spin states of each ligand and to their possible combinations with the iron spin [7]. The observed recombination kinetics can be influenced by

protein dynamics, if the intrinsic recombination rate constant is slower than these dynamics. By studies of viscosity and temperature dependence, the general averaged time scale for the recombination rate constant can be estimated [2, 7]. It is interesting to compare O_2, CO and NO molecules in this respect.

The CO recombination with heme is a single exponential process characterized by a slow rate constant $k \approx 10^6$ s^{-1} at ambient temperature and a low solvent viscosity (below viscosity of globin). This intrinsic (geminate) recombination rate is slower than both protein relaxation and CO escape, thus the recombination yield is very small (0.04) [7]. The rebinding of NO stable radical (S=S) is characterized by two-exponential kinetics with the rapid ($k_1 \approx 10^8$ s^{-1}) and slow ($k_2 \approx 5 \cdot 10^6$ s^{-1}) rate constants under ambient conditions [2]. Since the ground state heme has the quintet spin state (S=2), there are two starting states: $(S=2^1/_2)$ and $(S=1^1/_2)$ depending on mutual spin orientation of two species, while the recombination product has the doublet state $(S=^1/_2)$. Both type of geminate recombination require spin change; the slow process includes two step spin flip $(S=2^1/_2) \rightarrow (S=1^1/_2) \rightarrow (S=^1/_2)$, since spin-orbit coupling can mix states and induces spin transition with selection rule $\triangle S = 1$, and the rapid recombination occurs in one step $(S=1^1/_2) \rightarrow (S=^1/_2)$. The CO molecule is diamagnetic; all spins are paired, the total spin is zero. The heme-CO adduct is also diamagnetic (S=0). Since the ground state heme has the quintet spin state (S=2), the geminate recombination reaction is doubly spin forbidden. First it should be quintet — triplet transition, which needs to overcome an additional activation barrier (besides the spin flip, induced by SOC) and then a final triplet-singlet transition. This is the reason why the CO recombination with heme is so slow, in spite of the high binding energy [2].

Now we shall consider the dioxygen binding in more details. The ground state of the oxy-heme product is an open-shell singlet in agreement with EPR experiment and Messbauer spectra [2]. Thus the reaction of O_2 binding to heme is spin forbidden. At least the T-S transition has to occur [4].

Such spin flip can be induced by spin-orbit coupling (SOC) between the T and S states. One has to calculate the matrix element of the SOC operator [10-12]
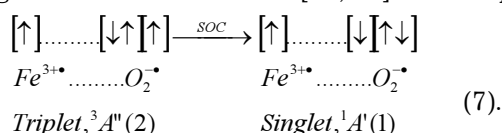
$$H_{SO} = \sum_A \zeta_A \sum_i \vec{l}_{i,A} \cdot \vec{s}_i = \sum_i \vec{B}_i \cdot \vec{s}_i = \sum_i (B_{i,x} s_{i,x} + B_{i,y} s_{i,y} + B_{i,z} s_{i,z}), \quad (6),$$

where ζ_A is a SOC constant for atom A ($\zeta_O = 153$ cm^{-1}), $\vec{l}_{i,A}, \vec{s}_i$ — are the orbital and spin angular momentum operators for the i-th electron, respectively. This is effective single-electron SOC approximation, which proved to be useful in many spectroscopic and chemical applications including spin-forbidden enzyme reactions [10-18].

Since the deoxyheme has a quintet ground state (four spins are unpaired, S=2), the adduct with the triplet dioxygen (two unpaired spins) would be expected to have either six (4+2=6) unpaired spins or two (4-2=2) unpaired spins depending on relative orientation of magnetic moments of the heme and O_2; the intermediate quintet spin state is also possible for the ground state spesies coupling. The triplet state of deoxyheme being very close in energy 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 the triplet O_2 in a spin-allowed oxyheme formation without spin flip. 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 [3, 6]. All other recombination processes include spin flip induced during heme — O_2 interaction; they seams to be more important for dioxygen binding [1-7]. It could start with the $^3A''(2)$ state, which is repulsive at shorter distance (R<2.5 Å) together with the septet $^7A''(1)$ state [3] (both are the ground state of the entrance channel heme + O_2 and go in parallel with some other multiplets until the short distances limit (2.5-3 Å). The energy gap is about 0.1 eV at these limits in agreement with Ref. [2, 3]. The optimized singlet ground state $^1A'(1)$ is lower in energy than other multiplets at least by

0.4 eV; this oxyheme product is an open-shell singlet of a complicated orbital and spin structure [3]. It has a short Fe-O distance (1.81 Å) [3] (reproduced in our DFT calculations, 1.84 Å) in contrast to the high-spin states (2-2.7 Å). Our result is close to the Fe^{3+}-O_2^- radical-pair structure in agreement with other DFT calculations and Weiss model [1-3, 8, 9]. The spin densities are equal to 0.94, -0.31, -0.72 for Fe-O-O chain, the bond angle is of 118°. The O-O bond distance (1.36 Å) and vibration frequency (1110 cm⁻¹) correspond better to superoxide ion [20]. (The closed-shell singlet has been obtained in a number of calculations [1, 19], however this result has been revised latter [8, 9].)

Account of our data and the results of Refs. [2, 3] allow us to consider the following scenario. At the intermediate distances 2.5-3 Å the starting $^3A''(2)$ state from the entrance channel transfers to the triplet Fe^{3+}-O_2^- radical-pair. In this region there are few crossing points between S and T states, including the $^3A''(2)$-$^1A'(1)$ states crossing, where spin change could occur [2, 3]. 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 (7). The two outer electrons of the ground triplet state dioxygen in two degenerate π_g-MO's provide a scheme [↑][↑]; electron transfer from Fe^{2+} to O_2 in order to produce the radical pair Fe^{3+}-O_2^- (7) can be accomplished by the occupation of either $\pi_{g,x}$- or $\pi_{g,y}$-orbitals. The both radical pairs could be in T and S 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 (7), since they correspond to the desired T→S transition and to the final product of the O_2 binding by heme. The scheme (7) is equivalent to the scheme (5) and the same explanation for the high SOC matrix element [10, 24] can be applied.

$$[\uparrow]........[\downarrow\uparrow][\uparrow] \xrightarrow{SOC} [\uparrow]........[\downarrow][\uparrow\downarrow]$$
$$Fe^{3+\bullet}........O_2^{-\bullet} \qquad Fe^{3+\bullet}........O_2^{-\bullet}$$
$$Triplet, ^3A''(2) \qquad Singlet, ^1A'(1) \qquad (7).$$

The starting triplet radical pair corresponds to a charge-transfer (CT) state described by $^3A''(2)$ wave function $^3\Psi_{CT_x} = \Re | (3d\alpha)(\pi_{g,x}\beta)(\pi_{g,x}\alpha)(\pi_{g,y}\alpha) |$, that is, transfer of an electron to the $\pi_{g,x}$-orbital of O_2 molecule, whereas the singlet radical pair

corresponds to a CT state described by $^1A'(1)$ wave function $^1\Psi_{CT_y} = \Re | (3d\alpha)(\pi_{g,x}\beta)(\pi_{g,y}\alpha)(\pi_{g,y}\beta) |$, namely, transfer of an electron to another degenerate orbital of oxygen, $\pi_{g,y}$ ($\Re$ means proper antisymmetrization of the wave function. The SOC arises between these CT states is the maximum possible for a system comprised of the light oxygen atoms [10]. The matrix element of the SOC operator (6) is equal to:

$$<^3A''(2)|H_{SO}^Z|^1A'(1)> = <^3_{CT_x}|H_{SO}^Z|^1_{CT_y}> = \frac{1}{2}<$$
$$_{g,x}|B_z|_{g,y}> = \frac{i}{2}_o = 76.5 icm^{-1} \qquad (8).$$

This SOC matrix element (8) is very close to a value of about 80 cm⁻¹, postulated in estimation of Landau-Zener rate constant for T-S transitions in spin-dependent O_2 and NO binding to heme [2, 21, 22]. With such a proposal for the generally unknown SOC integral [2] a quite reasonable estimation for the spin-dependent rate constants of the CO, O_2 and NO recombination in heme proteins are obtained [2, 21, 22]. For CO binding to heme a quintet-triplet-singlet stepwise transition is necessary which explains million times slower recombination rate in this case in comparison with the O_2 and NO recombination in heme proteins [2-4]. The gradient difference at the location of crossing points which enters the denominator of the Landau-Zener expression of the rate constant for spin transition is quite small (0.1-0.2 eV/Å) for O_2 and NO binding to heme [2, 3], thus the topology of the binding curves supports a rapid recombination of both ligands to hemo- and myoglobin.

The rapid NO rebinding to heme ($k_1 \approx 10^8$ s⁻¹) includes one-step quartet-doublet transition; since NO radical has one outer electron at degenerate π_x and π_y orbitals, a quite similar theory of SOC in quasidegenerate charge transfer states, like that, presented in Eqs. (4)-(5), (7)-(8), can be applied. The only deference is that the SOC integral (8) now includes π_x and π_y orbitals of NO molecule and thus is slightly smaller (about 60 icm⁻¹). The rate constant of spin transition in Landau-Zener model is determined by the square of the SOC integral. This explains that the rapid NO rebinding rate constant is about 3 times slower than the rapid rate constant of the O_2 recombination in heme proteins [2, 4].

All previous analysis of SOC effects in hemoproteins were based on assumption that the SOC integral in dioxygen binding to heme is determined by the iron ion and no attempt of direct calculation has been done [2, 21, 24]. As follows from our simple analysis, the SOC integral (8) is determined entirely by SOC in oxygen molecule and is connected with the degeneracy of two $\pi_{g,x}$ and $\pi_{g,y}$-orbitals in the open-shell of dioxygen. This enhancement of SOC effect by inclusion of charge-transfer to O_2 and involvement of superoxide-ion structure seams to be quite general in biochemistry [10]; it is applied also to those enzymes which have no transition atoms, like glucose oxidase, Eq. (4)-(5) [16, 17]. 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 [10, 23].

Conclusions. We have recalculated some potential energy surfaces (PES) cross sections for different multiplets along the heme-O_2 binding reaction coordinate in agreement with Refs. [1-3]. The Fe(II)porphine molecule (heme without side chains, shown in Fig. 1) coordinated with imidazol or ammonia molecules (Fig. 2, both are models of the proximal histidine) are used as in other similar studies [1-3, 8, 9, 19, 22]. The more realistic protoporphyrin IX model provides similar result for the ground state of the heme active site. Results of previous works [1-3, 8, 9, 19, 22] indicate that the main reason for the facilitated binding of O_2 to heme is a broad crossing region of the relevant spin states, which provides significant spin transition probabilities. They have shown that porphyrin is an ideal Fe(II) ligand for the spin-flip problem, because it tunes the spin states to be close in energy, giving parallel binding PES's, small activation energies and large transition probabilities in terms of the Landau-Zener approach [2, 3]. But none of these studies [1-3, 8, 9, 19, 22] have considered the reason for relatively large spin-orbit coupling, which induces the necessary spin flip in the heme-O_2 binding reaction; a general assumption that the SOC integral at Fe(II) ion of about 80 cm^{-1}, postulated in Ref. [21], have been used instead. We have shown that such SOC integral (8) is determined entirely by SOC in oxygen moiety and is connected with the degeneracy of two $\pi_{g,x}$- and $\pi_{g,y}$-orbitals in the open-shell of dioxygen. This is connected with charge transfer (CT) and with the Fe^{3+}-O_2^- radical-pair structure of the ground state ^{1}A'(1) and the close lying ^{3}A"(2) state near the crossing of the potential energy surfaces (PES) is presented in scheme (7). This scheme indicates that the triplet and singlet states, ^{3}A"(2) and ^{1}A'(1), differ by a single electron jump inside O_2 from the $\pi_{g,x}$ MO to the $\pi_{g,y}$ orbital. Such transformation is equivalent to the electronic orbital rotation, i.e. a torque, which creates transient magnetic field during the T-S transition and this magnetic field is responsible for the spin flip. In this model the magnetic perturbation occurs entirely in the oxygen moiety and the iron ion is silent.

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Спін-залежне зв'язування кисню з гемом і механізм підсилення спін-орбітальної взаємодії за рахунок переносу заряду

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Резюме. Вивчено спін-орбітальну взаємодію (СОВ) між початковим триплетним ^{3}A"(2) станом у вхідному каналі реакції зв'язування гем-O_2 і кінцевим синглетним ^{1}A'(1) станом з відкритою оболонкою, які визначені домінувальним вкладом структури радикальної пари Fe^{3+}-O_2^-. Перетини модельних поверхонь потенціальної енергії вздовж координати реакції для цих мультиплетів, розраховані за теорією функціоналу густини (ТФГ), узгоджуються з недавніми розрахунками ТФГ, відомими з літератури. Модель гема включає Fe(II)-порфін, координований з імідазолом або амоніаком у п'ятій координаційній позиції йона заліза, які моделюють гістидин як амінокислотний залишок міоглобіну. СОВ індукується головним чином на кисні за рахунок зміни орбітального кутового момен-

ту πg-оболонки при триплет-синглетному переході. Ця модель СОВ добре пояснює ефективну інверсію спіну в ході зв'язування гем-O_2.

Ключові слова: гемоглобін, міоглобін, цитохром оксидаза, спін-орбітальна взаємодія, триплет-синглетна спінова інверсія, структури радикальної пари, стани з переносом заряду, зв'язок двоатомних лігандів із гемом.

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