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REGULAR ARTICLE

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On the nature of oxoiron (IV) intermediate in dioxygen activation by non-heme enzymes

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Abstract We discuss electronic properties of the molecular systems with the short distance Fe–O unit, which are presumably formed as reaction intermediates during oxygen activation by non-heme enzymes. By performing an analysis of electronic densities in terms of multiconfigurational expansions of wavefunctions with localized orbitals the electronic properties of the Fe–O moiety in two model complexes are compared. The first one refers to the enzymatic intermediate, and the second biomimetic complex models a synthetic compound [Fe(O)(TMC)(NCCH$_3$)](OTf)$_2$ with a terminal Fe–O unit, which is experimentally characterized as the Fe(IV)=O species. We show that the orbital pictures of the FeO unit in both model complexes share common features. According to these simulations, the non-heme enzymatic intermediates may be assigned to the systems with the oxidation state of Fe between III and IV, as recently proposed for the TauD enzyme in experimental spectroscopic studies.

Keywords Quantum chemical modeling · Non-heme iron enzymes · Biomimetic complexes · Oxoiron intermediates

1 Introduction

Iron-dependent oxygenases, like cytochrome P450 [1–3] or non-heme iron-containing proteins [4–6], are among the enzymes responsible for activation and chemical transformation of molecular oxygen in organisms. Studies of structure, function and mechanism of action of iron-containing species controlling activity of genes under low oxygen conditions in cells are of a special importance for numerous applications, including rational drug design [7,8]. A complete picture of the entire reaction mechanism of oxygen activation at the mechanistic level is still a subject of discussion. Compared with the heme iron-containing species, the non-heme enzymes are considerably more complicated systems for investigations, because of additional flexibility of the ligand shell and presence of co-substrates in the coordination site of the metal ion [9,10]. By analogy with the heme proteins, the key role during dioxygen activation by non-heme monoiron enzymes is assigned to a possible reaction intermediate with the Fe–O moiety with a very short iron–oxygen bond. It is a matter of enlivening discussions on the chemical nature of this moiety in the non-heme enzymes with the dominating viewpoint that iron appears here in the oxidation state IV or even V [4–6,10–14].

Along with the studies of natural enzymatic systems, considerable efforts are being undertaken in the biomimetic field. The main efforts here are directed towards preparation and characterization of synthetic analogs of the enzymatic active sites of the high valent iron-oxo complexes with non-heme ligand sets. One of the most recent advances in this field describes the synthetic [Fe(O)(TMC)(NCCH$_3$)](OTf)$_2$ compound [TMC stands for 1,4,8,11-tetramethyl-1,4,8,11-tetraazacyclotetradecane, and OTf stands for CF$_3$SO$_3$] with a terminal FeO moiety [15,16]. This compound was characterized by Mössbauer, optical and magnetic circular spectroscopy, and its high resolution crystal structure revealed a short iron–oxygen bond length of 1.656(3) Å. By results of all these investigations, it was concluded that the iron–oxygen unit in this complex might be assigned to the type Fe^{IV}=O.

From theoretical perspectives, description of electronic properties of such systems presents a challenge for quantum chemistry. In particular, calculations of the electronic structure of [Fe(O)(TMC)(NCCH$_3$)]$^{2+}$ performed at the DFT level showed the charge population on iron close to +1 [16]. Difficulties in description of various valence or oxidations

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states of iron in typical oxo-iron complexes by using standard quantum chemical methods have been pointed out some time ago, for example, by Siegbahn and Crabtree [17]. By using DFT type of calculation they analyzed electronic distributions for several five- and six-coordinate iron complexes ($Fe(H_2O)_6^{2+}$, $Fe(OH)_3(H_2O)_3$, $Fe(H_2O)_6^{3+}$, $Fe(OH)_4(H_2O)_2$, $Fe(OH)_4(NH_3)_2$, $Fe(OH)_4(NHCH_2)_2$) with different oxidation states II, III, and IV, and found that "from a normal chemical point of view" the computed populations were "quite surprising." Namely, in all cases the charge population on iron (around +1) was much smaller than expected from a literal interpretation of the oxidation state and did not even increase with the oxidation state. Also, in all cases, the 3*d* population on iron was quite stable slightly above 6. The same discouraging conclusion about the ability of standard quantum chemistry methods to reproduce "chemical" interpretation of electronic structure of metal ions in certain oxidation states in oranometallic complexes may be traced in many publications, including those referring to metal porphyrins [18]. To better distinguish between various oxidation states, it was suggested to analyze spin densities on metal following results of DFT [17] or more advanced [19] calculations or simply correlate oxidation states with equilibrium bond lengths. The necessity of reliable quantum chemical identification of various oxidation states of metals becomes an important issue, considering the growing usage of quantum-based methods, including hybrid QM/MM methods, in studies of metallopoteins.

Concerning the electronic properties of the Fe^{IV}=O unit, appearing in the reaction intermediate during the cytochrome P450 catalytic cycle (so-called Compound I), Meunier et al. [1] suggest the following bonding picture: the FeO moiety has the same bonding as the O_2 molecule – one filled σ-FeO orbital, two filled π-FeO orbitals, and two singly occupied π^*-FeO orbitals. Further, "with this electronic structure, Fe has formally four electrons (two in a δ orbital, the pure d_{x2-y2}, and two in the π^*-FeO orbitals that are considered as the d_{xz} and d_{yz} iron orbitals) and is hence Fe(IV)." A related molecular orbital picture, describing the Fe–O double bond, but in the biomimetic non-heme iron complex $[Fe(TPA)OOH]^{2+}$, where TPA stands for tris(2-pyridylmethyl)amine, was discussed in the paper of Bassan et al. [20]. The authors considered the electronic structure of the Fe^{V}=O unit as σ^2 (FeO) π^4 (Fe–O) π^{*2} (FeO) $3d^1$ (Fe) and, according to this representation, Fe has formally only three electrons, and it is assigned to the oxidation state V.

It should be noted, however, that such quantum chemical interpretations did not appear as the results of analyses of computed electron densities, but deduced on the base of "bonding principles" [21]. In this communication we attempt to address the subject of the electronic structure of the oxo–iron intermediate in dioxygen activation by non-heme enzymes from another theoretical perspective. We propose to analyze multiconfigurational expansions of wavefunctions of the model compounds, which mimic non-heme enzymatic intermediates with the Fe–O moiety, computed at the multiconfigurational self-consistent field (MCSCF) level of the theory [22]. The application of strongly localized orbitals in such multiconfigurational expansions allows one to simulate valence bond picture of the molecular system and to deduce the bonding properties directly from computed wavefunctions. The MCSCF method is used here not as a tool to calculate properties of the compounds under study, but as a tool to construct mimics of valence bond expansions as shown in the paper of Nemukhin and Weinhold [23].

We will use the biomimetic complex $[Fe(O)(TMC)(NCCH_3)](OTf)_2$ [15,16] as a reference structure, namely, as a structure in which the presence of the Fe^{IV}=O unit is supposed to be proved. For comparison we analyze the model intermediate (MODI) occurring in the reactions of oxygen transformations catalyzed by the hypoxia-inducible factor asparaginyl hydroxylase (FIH-1). This enzyme activates dioxygen molecule and, through the chain of chemical transformations, finally performs hydroxylation of the C–H bond of an asparagine residue of the C-terminal transactivation domain (CAD) of hypoxia-inducible factor HIF-1α. These reactions are of extreme importance for studies of hypoxia-related processes in cells [24], and characterization of possible intermediates (including those with the active Fe–O unit) is necessary for understanding the reaction mechanisms. The crystal structure of FIH with the 2-oxoglutarate (2OG) obtained under anaerobic conditions and solved at the resolution 2.25 Å has been recently reported (PDBID: 1H2L) [25]. This provides us a reasonable starting arrangement of atoms in the ligand shell around iron in the native enzymatic system.

2 Calculations

Supporting information to the publication [16] allowed us to reproduce some results of DFT calculations for the biomimetic complex $[Fe(O)(TMC)(NCCH_3)]^{2+}$. Compared with the initial structure [16], we constructed a slightly simplified model (Fig. 1) abbreviated in this work as synthetic compound (SYNC). Calculations in Ref. [16] have been carried out by using the local density approximation of Vosko–Wilk–Nussair together with gradient corrections for exchange (Becke88) and correlation (Perdew86).

We used the B3LYP calculations with a slightly modified LANL2DZdp basis set [26] (diffuse functions were kept on the oxygen atoms only) and the Gaussian03 program system [27] calculations to obtain equilibrium geometry configurations in the triplet and quintet states. The results were essentially the same as cited in Ref. [16]. Exactly, as in Ref. [16], our DFT calculations predicted the triplet state $S = 1$ being the ground electronic state, while energy of the quintet state $S = 2$ was about 13 kcal/mol higher. As discussed in Ref. [16], it is reasonable to assume that this conclusion results from deficiencies of the DFT method, and actually the quintet state must be considered as the most likely candidate for the ground state.

Second, the MODI species for the reaction $Fe^{2+} + O_2$ in the FIH protein environment has been created. Starting from the relevant crystal structure (PDBID: 1H2L [25]) and adding

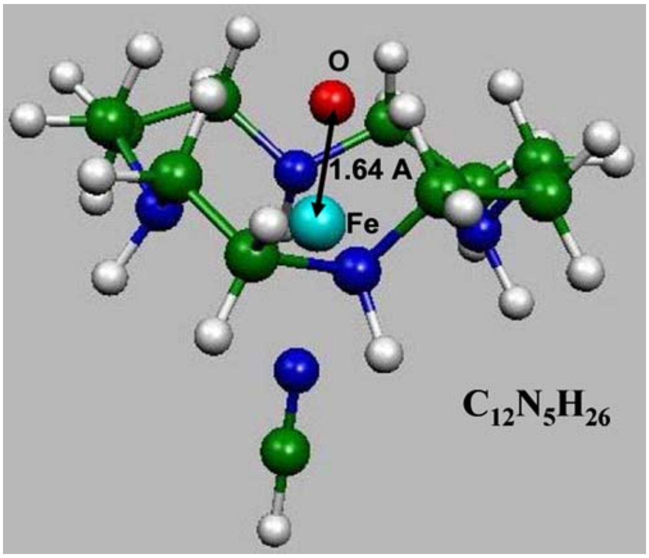

Fig. 1 Structure of the Synthesis Compound (SYNC) complex, constructed by motifs of $[Fe(O)(TMC)(NCCH_3)]^{2+}$ [16]. Distances from iron to five nitrogen atoms are within $2.11 \div 2.15$ Å

the oxygen molecule, the model system, composed of the iron ion, side chains of His and Asp residues, a simplified 2OG species and O_2, has been constructed. Equilibrium geometry configurations (shown in Fig. 2) have been optimized again by using B3LYP/LANL2DZdp method. Several spin states compete for the ground electronic state of the system. The lowest energy configuration corresponds to $S = 3$, next follows the state with $S = 2$, which is 10.7 kcal/mol higher in energy. As follows from several DFT calculations of the energy profiles for the reactions of oxygen activation by non-heme biomimetic complexes [28,29], as well as from our calculations, immediately in the entrance channel there should be a change in the spin state of the system: $S = 3 \rightarrow S = 2$. Therefore, in Fig. 2, we show the equilibrium geometry structure corresponding to the $S = 2$ state, which apparently may be considered as the reagents configuration for the forthcoming transformations.

The first stage of the reaction consists of activation of the dioxygen molecule followed by interaction of activated oxygen with the 2OG analog giving rise to the CO_2 species and the corresponding replacement in the ligand shell of iron. The computed reaction mechanism leading to the intermediate with the Fe–O moiety will be described elsewhere, and here we concentrate mostly on the features of the intermediate. The computed structure for this intermediate is shown in Fig. 3. Calculations at the B3LYP/LANL2DZdp level show that the lowest energy electronic state in the corresponding equilibrium configuration refers to the quintet spin state $S = 2$.

Further simplification of this model was necessary in order to perform expensive calculations at the MCSCF/LANL2DZdp level. The most important features of the ligand shell around the iron ion should be kept. Therefore, we replaced the imidazole rings by the NH_3 moieties and simplified the Asp side-chain and the remaining fraction of the 2OG analog by the HCO_2^- fragments. Equilibrium geometry configuration of this system was additionally optimized at the B3LYP/LANL2DZdp level. Thus obtained "MODI" system is illustrated in Fig. 4.

Comparison of "SYNC" and "MODI" structures (Figs. 1, 4) shows that in both cases the Fe–O unit possesses a very short bond length (1.64 Å in SYNC, 1.61 Å in MODI); however, the ligand shells are different. Our reasoning is as follows: if SYNC is assigned to the structure with the Fe^{IV}=O

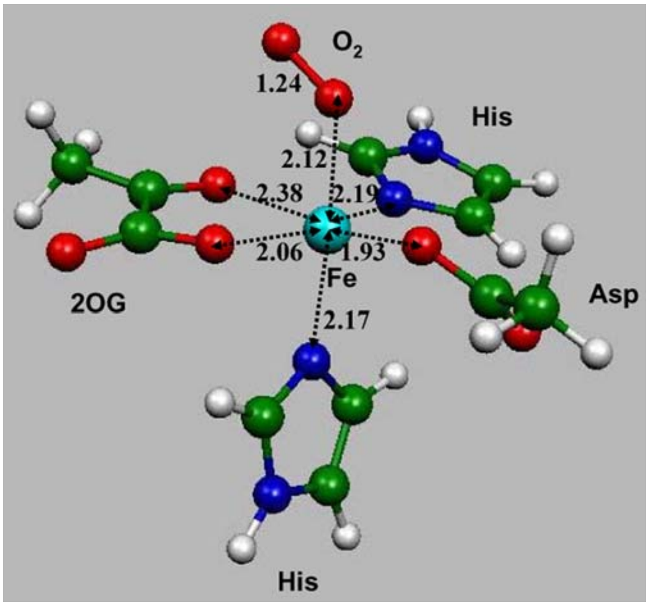

Fig. 2 Structure of the model oxygen molecule–iron site complex, constructed by motifs of PDBID: 1H2L [25]. Distances are shown in angstroms

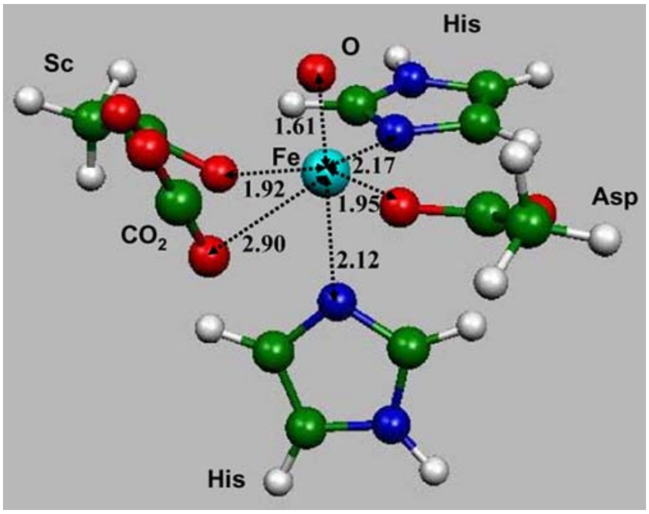

Fig. 3 Structure of the model intermediate with a short (1.61 Å) Fe–O bond as obtained from the reagents (Fig. 2). Distances are shown in angstroms

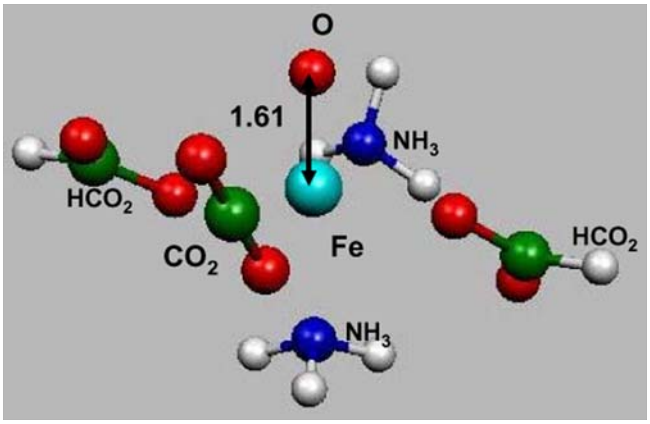

Fig. 4 Structure of the model intermediate (MODI) complex, which mimics the intermediate shown in Fig. 3. Distances from iron to nitrogen atoms from NH_3 are 2.13 and 2.18 Å, and to oxygen atoms from the HCO_2 units are 1.91 and 1.98 Å

moiety [15,16], then a direct comparison of the electronic properties of MODI to those of SYNC may help to understand the nature of the intermediate in the FIH-catalyzed process, what is a major subject of our concern.

In order to compare the local properties of the Fe–O unit in both systems, we apply the following strategy. In both cases we computed the natural bond orbitals (NBOs) [30] for the quintet states of the systems, and introduced these highly localized orbitals to the multiconfigurational expansions of the complete active space SCF method. Such a procedure allows one to mimic the valence bond type wavefunction and to recognize the major contributions to the electronic structure in terms of localized elements [23]. All calculations have been performed with the LANL2DZpd basis set (and the corresponding pseudopotential for iron) [26] by using GAMESS(US) software [31]. In both cases we included in the active space the initial orbitals (NBOs) referring to the $3d$ orbitals of iron and $2p$ orbitals of the oxygen atom (the NBO analysis performed with the Hartree–Fock electronic densities did not show bonding orbitals for the Fe–O unit). In both cases we distributed 12 active electrons over 9 active orbitals, which amounted to 1050 configuration state functions. In the course of orbital optimization during the MCSCF calculations certain orbital mixing occurred, but the localized nature of the MCSCF orbitals was kept, which allowed us to analyze the electronic structure in the valence bond-like fashion.

The results of MCSCF calculations for the SYNC and MODI systems turns out to be very interesting. First, these calculations for SYNC show that unlike in the DFT computations, the quintet state ($S = 2$) is really lower in energy that the triplet state. Therefore, our calculations provide a support to the considerations and assumptions reported in Ref. [16] for the nature of the ground electronic state of $[Fe(O)(TMC)(NCCH_3)]^{2+}$. Correspondingly, the quintet state for MODI is also the lowest one among the triplet, quintet, and septet states.

Second, an analysis of the multiconfigurational expansions with the localized MOs shows that the electronic structure of the Fe–O moiety in SYNC closely resembles that in the heme-like cytochrome enzyme [1,21]. We can distinguish one filled σ(FeO) orbital, two filled π(Fe–O) orbitals, two singly occupied π*(FeO) orbitals, and two singly occupied $3d$(Fe) orbitals in the electronic configuration entering the multiconfigurational wavefunction with the coefficient 0.86:

$$\begin{aligned}&\Psi(\mathrm{SYNC})\\&\quad = 0.86\{\sigma^2(\mathrm{FeO})\pi^4(\mathrm{Fe\text{–}O})\pi *^2\,(\mathrm{FeO})3\mathrm{d}^2(\mathrm{Fe})\} + \cdots .\end{aligned}$$

Following the consideration presented in Ref. [1] for the iron-oxo species in cytochrome, such electronic configuration allows one to assume a double-bonded cartoon Fe=O (as in the O_2 molecule), and the assignment of formally four electrons (two in the $3d$-orbital and two in the π*(FeO)-orbitals) to iron, which corresponds to the oxidation state IV. Thus, an analysis of the electronic structure of the Fe–O moiety in SYNC is qualitatively consistent with the conclusions of Ref. [16], assuming the Fe^{IV}=O unit.

For the MODI structure the analysis was performed exactly in the same fashion as for SYNC. We used MOLEKEL software [32] for graphical representation of the orbitals. By viewing them, we could recognize the same pictures of the MCSCF orbitals. Figure 5 compares the key orbitals for SYNC and MODI. However, the composition of the multiconfigurational wavefunction for MODI is different:

$$\begin{aligned}&\Psi(\mathrm{MODI})\\&\quad = 0.66\{\sigma^2(\mathrm{FeO})\pi^2(\mathrm{Fe\text{–}O})\pi *^2\,(\mathrm{FeO})3d^2(\mathrm{Fe})\}\\&\qquad +0.37\{\sigma^2(\mathrm{FeO})\pi *^2\,(\mathrm{FeO})3d^2(\mathrm{Fe})\sigma *^2\,(\mathrm{FeO})\} + \cdots .\end{aligned}$$

Only one doubly occupied π(Fe–O) orbital is seen, and second, the weight of the electronic configuration with the occupied σ(FeO), π(Fe–O), π*(FeO), $3d$(Fe) orbitals is considerably lower (0.66 vs. 0.86). A large mixture of the configuration with the filled antibonding orbital σ*(FeO) instead of the bonding species π(Fe–O) is found in the wavefunction.

We can interpret the results of the calculations as follows. The properties of the Fe–O moiety in the MODI system share certain common features with those of Fe–O in SYNC with respect to population of the key orbitals π(Fe–O), π*(FeO), $3d$(Fe). The computed occupation numbers of the orbitals π(Fe–O) and π*(FeO) were 2.0 and 1.6, respectively, in both compounds. Compositions of these orbitals in terms of the initial iron and oxygen orbitals resembled the assignment described by Meunier et al. [1]. Namely, the π*(FeO) orbitals contained essential contributions from the $3d$ iron atomic orbitals, while the bonding π(Fe–O) orbitals were almost equal mixtures of iron and oxygen orbitals. However, the bond order of FeO in MODI should be lower than two and, correspondingly, the Fe–O bond should be weaker in the MODI complex, in spite of the observation that the Fe–O bond length in MODI is shorter that in SYNC. According to our analysis, there is no indication of occurrence of iron in the oxidation state V (as suggested in Ref. [20]), which would require electronic configurations with a singly occupied $3d$(Fe) orbital.

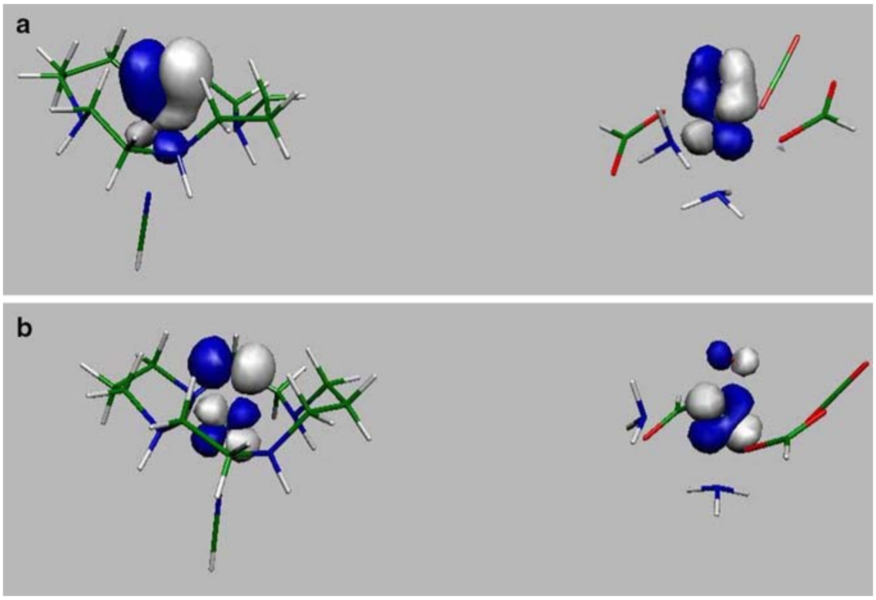

Fig. 5 Comparison of the orbitals from SYNC (*left*) and MODI (*right*) complexes: **a** π(Fe–O), **b** π^*(Fe–O). The *graphs* have been created by using MOLEKEL [32]

3 Discussion and conclusions

An involvement of elusive oxo-iron (IV) intermediate in the reaction cycle of non-heme monoiron oxygenases is still an assumption following the heme paradigm. The species with the highly reactive Fe=O moiety have been experimentally characterized in cytochrome P450 [1–3] or biomimetic complexes [15,16]. Currently considerable efforts are undertaken to detect evidences of this high-valent iron intermediate by using Mössbauer spectroscopy [12,13] or EXAFS spectroscopy [14] for one of the non-heme enzymes, TauD. The EXAFS data demonstrated the presence of a short (1.62 Å) distance between the iron center of the reaction intermediate and one of its ligands, and the conclusion was that, most likely, the corresponding peak referred to Fe=O. The XANES spectra and the high Mössbauer isomer shift indicated that the oxidation state of iron in this intermediate might be between +III and +IV. Experimental investigations of this "intriguing possibility" are ongoing [14]. Significance of an analysis of oxidation state of iron is due to its direct relation to the details of reaction mechanism described in usual chemical terms.

The purpose of this study was to clarify the nature of Fe(IV)=O moiety in the crucial reaction intermediate of the non-heme catalytic cycle by using computational methods of quantum chemistry and to encourage (or discourage) experimental efforts in locating the corresponding compounds. In introduction we discussed the difficulties of using usual population analysis for this goal. Therefore, we applied another methodology: let us compare, within the same quantum chemical approximation, electronic properties of the FeO unit in the molecular system of principal interest, that is, for the environment typical for non-heme active site, to electronic properties of the similar unit in the reference molecular system with known or at least commonly accepted features. Since all experimental evidences and also chemical knowledge indicate that the synthetic analog [Fe(O)(TMC)(NCCH$_3$)](OTf)$_2$, the precursor of SYNC, is best described as the complex with the Fe^{IV}=O moiety [15,16], then it would be reasonable to use it as a reference system. In order to distinguish electronic properties of the FeO unit inside larger molecular systems, SYNC and MODI, we analyzed multiconfigurational expansions of their wavefunctions with localized orbitals.

Our analysis shows that the possible intermediates in the reactions catalyzed by non-heme enzymes with the short Fe–O bond can actually be considered as the species with Fe in the oxidation state IV, at least on the same grounds as this refers to [Fe(O)(TMC)(NCCH$_3$)](OTf)$_2$.

We should emphasize that such an analysis is beyond an academic exercise in terminology. The reaction of dioxygen activation by Fe(II)-containing enzymes apparently starts by donation of electron from iron to oxygen molecule. From the chemical point of view the successive steps of the reaction assume further changes of the oxidation state of iron up to IV, or even V. If in theoretical consideration by means of quantum chemical tools one obtains that the population analysis does not predict changes in charge population of metal for different oxidation states, then these simulations may be a subject of certain criticism. In the MCSCF calculations performed here for SYNC and MODI systems we see dominant configurations with the charge population on iron +3 (the overall natural atomic population analysis with the MCSCF wavefunctions shows values between +2 and +3), and small mixtures of the configurations with the formal charge on iron +4. This conclusion based on theoretical modeling correlates well with the experimental results [14], which suggest an intermediate oxidation state of iron in TauD between III and IV.

If reliable calculations of properties of these compounds are required at the quantitative level, but not an analysis of their electronic structure, a choice of the method should be different. It is known that CASSCF approximation faces a problem for transition metal complexes due to lack of dynamic correlation [33]. Successful applications of the DFT technique in this field are presented in the literature [28,29,34,35]. An interesting discussion of the subject is given in the paper of Ghosh and Taylor [36].

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