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SPIN AND ELECTRON DENSITY REDISTRIBUTION UPON BINDING OF NON-INNOCENT LIGAND BY IRON IN ENZYMATIC ENVIRONMENT: CHALLENGES FOR QUANTUM CHEMISTRY

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Abstract: The quality of the description of a chemical bond between the metal (active site) and the ligand (substrate) critically depends on the electronic processes accompanying the bond formation. However, as far as transition metal centers (TM) in enzymes are considered, most of the properties related to their electronic structure are extremely challenging for quantum chemistry. Especially severe problems appear for the bonding of NO to ferrous sites, *e.g.* in myoglobin or non-heme enzymes. Therefore, special care has to be shown in the assessment of a quantum chemical method employed with respect to its power in describing the properties of interest. In this work we discuss spin-resolved Fe-NO charge transfers and their relation to the metal spin state, with special attention paid to the interpretation of the bonding between NO and the transition metal center in terms of dative or covalent contributions; furthermore, the impact of spin and the electron transfer on the reactivity of the center is discussed. The stress is put on the role of the coordinating environment in controlling the reaction mechanism via fine-tuning of the spin and the oxidation status of the metal core. This goes in line with the high significance of spin in enzymatic reaction mechanisms (*cf.* multi-state reactivity proposed for iron enzymes).

Keywords: NO ligand, Fe^{II} center, DFT, electron density transfer channels

1. Introduction

This contribution addresses problems intrinsic to the description of electronic properties for large ensembles of nuclei and electrons (*e.g.*, bio-and

biomimetic systems). Practical quantum chemistry, although rooted in first-principles quantum mechanics, suffers from the separation of nuclear and electronic motions within Born-Oppenheimer (B.-O.) approximation. Therefore, robust quantum-chemical methods (like the density functional theory, DFT) offer merely B.-O. ground-state energy hypersurfaces. They in turn may serve to simulate the dynamics of "heavy" nuclei, however, the quality of any molecular dynamics simulations strongly relies on the quality of the force field derived from the energy hypersurface. Thus, we should be aware that the so called *ab initio* simulation procedures do not provide a universal remedy for problems encountered in theoretical approaches to the structure and properties of biological systems which launches a quest for phenomenological approaches and parameter-based procedures, rooted in deep understanding of physics behind the processes in question. This adds to the uncertainties related to the choice of the quantum chemical protocol *per se*, *e.g.* the selection of a proper exchange-correlation functional in DFT. The functional should be capable of consistently describing the diversity of physical interactions behind the collective properties of interest in biosystems: at this point such phenomena as hydrogen bonding or dispersion interactions should be highlighted. Therefore, employment of quantum chemical methods requires experience and good knowledge of the involved physics. More discussion on our experience in quantum chemical methods used in the field and representative examples for quantum chemical (QC) modeling of the reactivity of enzymatic active sites are given in recently published mini reviews [1, 2].

Even if B.-O. approximation may suffice in a number of molecular problems, many properties strictly related to the electronic structure of transition metal sites (TM) in enzymes are extremely challenging for quantum chemistry. Especially severe problems appear for the bonding of NO to ferrous centers, *e.g.* in myoglobin or non-heme enzymes: it may serve as a good case study since the NO bonding is both an intriguing and important feature of enzymatic active sites as it presents a seemingly simple property (a well isolated bond between two subsystems) but difficult to treat with sufficient accuracy by QC methods. The quality of the metal–ligand bond description depends critically on the electronic processes accompanying the bond formation which, in turn, rely on a proper description of the electronic structure. NO is a non-innocent, redox-active ligand which may donate or withdraw an odd electron to/from the iron centre along with Fe-N-O bond sequence, as well as covalently interact with the transition metal ion. Three major valence structures are proposed for $\{Fe^{II}NO\}^7$ complexes (where $6d$ electrons from the valence shell of Fe^{II} are counted jointly with an odd electron on NO), namely $Fe^{II}NO^0$, $Fe^{III}NO^-$ or $Fe^{I}NO^+$. However, this scheme is still oversimplified as partial decoupling of spins in the electron pair forming the polarized covalent bond may perturb the donation – backdonation processes. On the other hand, the selection of a proper valence structure is seemingly of vital importance since the oxidation status and the spin on the metal critically influence its reactivity and the strength of the bond (being an important parameter in any force filed).

In the following section we critically discuss the way of extracting detailed information on the Fe-NO bond characteristics, based on designed analysis of the electron density deformation upon the formation of the bond between the ligand (NO) and the ferrous center (Fe^{II}). The decomposition of the global density deformation into orbital- and spin-resolved independent contributions is done by the analysis of one-electron charge transfer channels between the metal and the ligand, within Natural Orbitals for Chemical Valence (NOCV) approach [3–5]. A precise description of the ground-state electronic structures of the complexes is a prerequisite to extract meaningful mapping of electron density redistribution upon fragment bonding onto one-particle contributions. For non-innocent systems this may require the involvement of higher level correlated wave function methodologies to validate the DFT results (indispensable for presumably multiconfigurational characteristics that could violate the Kohn-Sham assumption [6, 7]). Here we are presenting the results of DFT modeling with the calculation scheme set on the basis of CASPT2 (complete active space multiconfiguration approach). The interpretation of the bonding between NO and a transition metal center is given in terms of dative or covalent contributions; furthermore, the role of the coordinating environment in controlling the reaction mechanism via fine-tuning of the spin and oxidation status of the metal core is highlighted.

2. Critical selection of quantum chemical methodology and molecular models

As already stated, quantum chemistry may provide insights into the electronic structure and properties of non-innocent systems comprising transition metals. This is of special value since, unlike experiment, QC offers direct and unbiased access to the electronic structure of transition metal sites, not only for stable (and thus experimentally well characterized) species, but also for elusive active forms and even for transition states of chemical reactions. Therefore, in spite of serious limitations discussed in the Introduction, QC has become a useful complementary tool for bio-inorganic chemistry of today. Most massive quantum chemical calculations are nowadays carried out with density functional theory (DFT) methods, for their reasonable compromise between the accuracy of the obtained results and the needed computational resources. DFT is especially attractive as it (in principle) offers a straightforward route to the direct observable, total electron density. However, since transition metal sites are well recognized as "difficult cases" for QC, large errors may be occasionally encountered. Even if the quality of the results can be frequently improved by making the model more realistic, *e.g.* increasing the system size, accounting for long-range interatomic forces and thermodynamics, performing statistical sampling or molecular dynamics, there exist intrinsic problems with the electronic structure of transition metal systems (*e.g.*, variable spin states on the metal, spin coupling, nondynamical correlation), not always satisfactorily tractable within DFT methodology. Here, the

ab initio wave function approach may help by systematic treatment of the electron correlation, however, for a price of much higher computational complexity which makes their applications to sufficiently large, biologically relevant models unpractical. Nevertheless, *ab initio* methods may help to select the proper DFT scheme, with the best selection of the exchange and correlation functional from a broad manifold of available potentials, designed specifically to cover *e.g.* dispersion, H-bonding, left-right correlation in atypical bonds, etc. [8].

Another point is the selection of a molecular model for the system of interest. A whole enzyme or an extended active site region is hardly tractable by quantum chemistry and therefore the model must be considerably reduced, unless a hybrid quantum mechanics / molecular mechanics (QM/MM) method is selected where the active site of the protein is described by a QC method and the remaining part of the system by molecular mechanics [9]. The first limitation concerns the size and composition of the QC model of an active site, which is usually a compromise between its completeness and the computational cost, growing very fast with the model size. In our example, a minimal model would include but a Fe^{II} center and its first coordination shell with properly truncated protein residues (*e.g.*, hydrogen saturation of the cut bonds, fixing coordinates of boarder atoms; see Figure 1 for an illustrative example).

For the properties under investigation in this work, the selection of structural models and the choice of the appropriate quantum chemical method must be accompanied by an additional degree of freedom: proper selection of the electronic

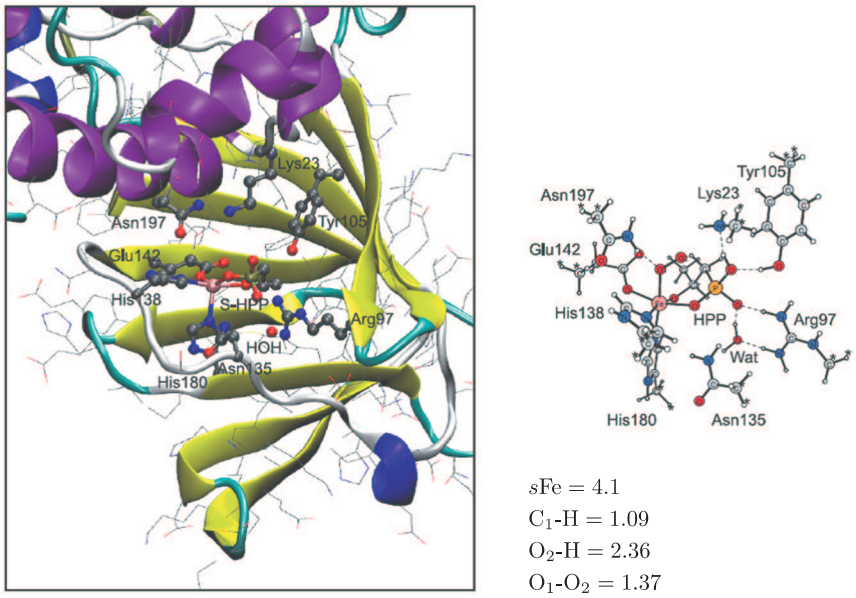

Figure 1. Active site region of X-ray HppE-Fe^{II}-HPP (PDB: 1ZZ8 [10]) structure and its model (with bound O_2) used in QC investigations of the catalytic reaction mechanism (after ref. [11]); asterisks mark atoms with fixed coordinates

configuration. The present contribution is focused on the electronic structure of nitrosyl complexes to the iron(II) center, either in the porphyrin-type or pentaaqua iron(II) architecture, a representative of inorganic mimics. Iron porphyrin systems serve as models of active sites in iron-containing heme enzymes which form a large and important group of metalloproteins involved in many processes, like transport and activation of small inorganic ligands (oxygen, nitric oxide), oxygenation and degradation of organic molecules (including drugs), as well as in electron transfer processes. All the results were obtained from unrestricted DFT (UDFT) calculations to obtain appropriate electron densities, with the def2-TZVP basis set and a BP86 functional available in the Turbomole 5.9 package [12]. The choice of a proper functional was based on extensive testing of the DFT performance over a range of $\{\text{Fe-NO}\}^7$ complexes against multiconfigurational wavefunction methods [4, 13]. The previous *ab initio* results have indicated that non-hybrid functionals, such as BP86, reasonably reproduce CASSCF electron spin densities for these demanding, open-shell systems (ref. [4]).

The structures of two representative $\{\text{FeNO}\}^7$ complexes (shown in Figure 2) are taken from the reference [4]. The 6-coordinated Fe^{II}-porphyrin model, $\text{FeP}(\text{NH}_3)\text{NO}$ (Figure 2 a), was constructed under C_s symmetry (mirror plane xz is specified with the bent Fe-N-O motif, the four equatorial Fe-N bonds lying approximately on the diagonals of the xy plane). The $\text{Fe}(\text{H}_2\text{O})_5\text{NO}^{2+}$ complex with the linear Fe-N-O motif (Figure 2 b) has actual C_{2v} symmetry, though only slightly distorted from an ideal C_{4v} one (with N-O and Fe-O along the z axis, the remaining Fe-O bonds lying on the xz, yz mirror planes). The ground spin states were taken for the both models, *i.e.* doublet ($S = 1/2$) for $\text{FeP}(\text{NH}_3)\text{NO}$ and quartet ($S = 3/2$) for $[\text{Fe}(\text{H}_2\text{O})_5\text{NO}]^{2+}$.

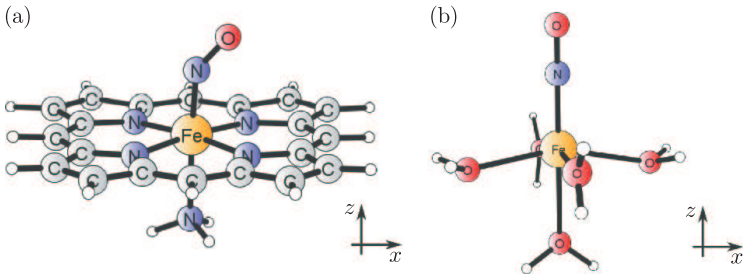

Figure 2. Structures of studied complexes, optimized at BP86/def2-TZVP level: $\text{FeP}(\text{NH}_3)\text{NO}$ (a) and $\text{Fe}(\text{H}_2\text{O})_5\text{NO}^{2+}$ (b) (hydrogen in open circles, after [5])

The SR-NOCV analysis is focused here at the bond formation between the two open-shell fragments, eventually making the open-shell complex. Good representation of electron densities both of the complex and the promolecule (made of respective fragments in the geometry they take in the complex) requires an adequate choice of electronic configurations of the fragments. This may be not straightforward since the fragments frequently do not represent stable moieties characterized by experiment; we comply to the presumption that spin density

distribution in open-shell fragments should resemble the electronic situation in the molecule as closely as possible. To obtain meaningful electron transfer channels, we based the pre-selection of appropriate configurations on the Fe and NO fragments upon the character of natural spin orbitals (eigenvectors of spin density matrix) for the two complexes. Detailed inspection of spin densities of the complexes and appropriate fragments [5] yielded the picture of the $Fe^{II}P(NH_3)NO$ ($S=1/2$) adduct as built of NO^0 ($S=1/2$) and $Fe^{II}P(NH_3)$ ($S=0$) fragments; $[Fe^{II}(H_2O)_5(NO)]^{2+}$ complex was described as NO^0 ($S=1/2$) antiferromagnetically coupled to $Fe^{II}(H_2O)_5$ ($S=2$) fragment.

3. Independent charge transfer channels and the characteristics of the Fe-NO bond

As already stated before, the diagonalisation of the differential electron density for each complex provides NOCV orbitals. The pair of the corresponding NOCVs (with a negative eigenvalue depicting a decrease, and a positive eigenvalue – an increase in the electron density), defines an electron transfer channel between the two fragments. The eigenvalue offers a measure for electron density transferred along a particular electron transfer channel, while the plot visualizes the pathway of the electron density transfer (from the region depleted to the region enriched in electron density). Plots are generated with the Gabedit software [14] from the output of the Natorbs [15] utility, serving to calculate the NOCV orbitals. The independent electron flow channels, plotted according to the convention ascribing red contours to the depletion and blue contours to the gain of electron density, are shown in Figures 3 and 4 for $Fe^{II}P(NH_3)NO$ and $[Fe^{II}(H_2O)_5(NO)]^{2+}$, respectively. Each channel may be interpreted as the electron flow from the red area to the blue region. The corresponding eigenvalue modulus is given together with the contour plot for each channel and serves for estimating the number of electrons redistributed along this channel.

Contour plots of the four dominant electron density flow channels obtained for the $FeP(NH_3)$-NO adduct, differing in spin (α or β) and in the plot plane (xz or yz) are shown in Figure 3, together with their corresponding eigenvalues. The two channels in the xz plane (comprising the Fe-N-O bond) have distinct characters for α and β spins. The α-spin component (upper left panel) may be interpreted as inter-fragment delocalization (partial σ-donation to the Fe-NO bonding region) of the unpaired electron from the π_x^* on NO to the empty Fe d_{z2}. This is accompanied mainly by intra-fragment polarization of the β-spin density that negligibly contributes to the inter-fragment electron and spin transfer. On the contrary, the pair of channels in the yz plane (Figure 3, right panel) have the same character (π^*-backdonation from the Fe d_{yz} to the empty π_y^* orbital on NO) and comparable eigenvalues (0.43, 0.46) for both spins. Effectively, the 0.9 electron becomes transferred from Fe to NO via these channels. They correspond to the Fe $\rightarrow$ NO π^*-backdonation (making the dative bond) and do not contribute to the net spin transfer.

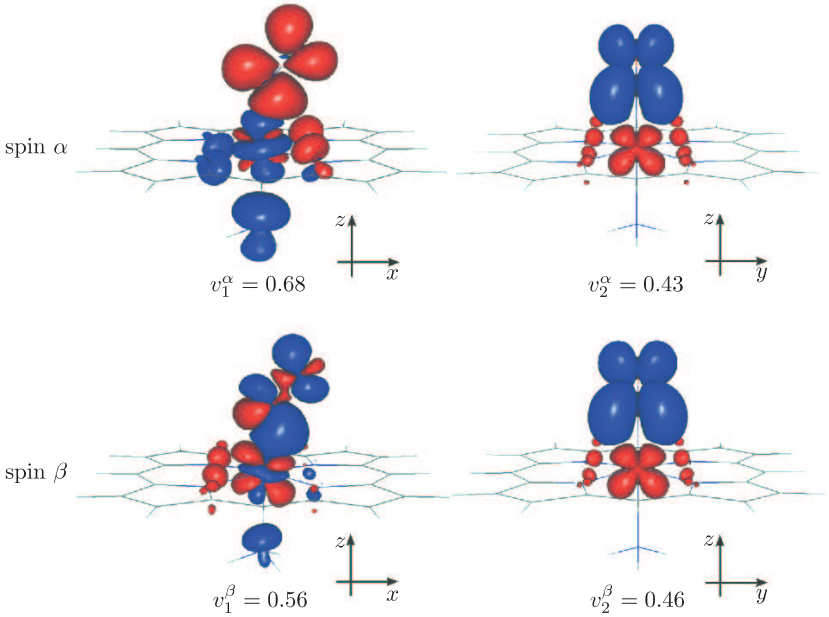

Figure 3. Contour plots of most important electron density transfer channels for $FeP(NH_3)(NO)$ complex (with eigenvalues > 0.2); blue: electron inflow, red: electron outflow, contour value: 0.001 au)

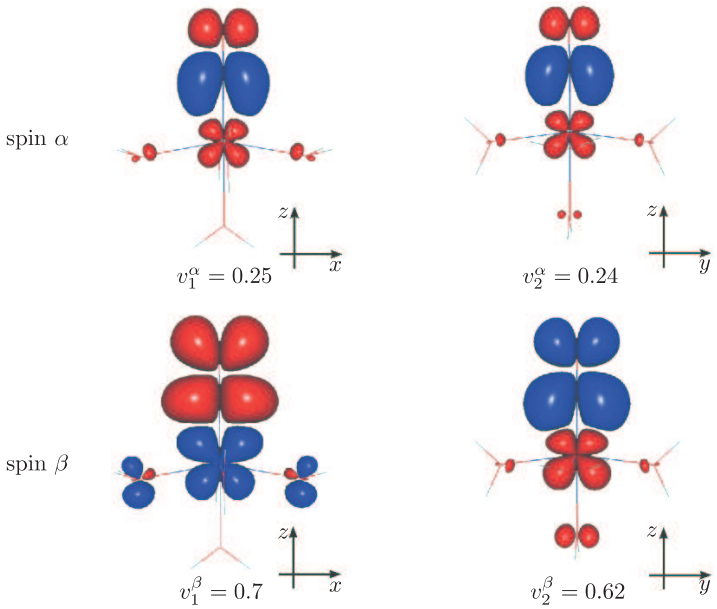

Figure 4. Contour plots of most important electron density transfer channels for $[Fe^{II}(H_2O)_5]^{2+}$-NO complex (with eigenvalues > 0.2); blue: electron inflow, red: electron outflow, contour value: 0.001 au)

The contour plots of the four dominant electron density flow channels for the $[Fe^{II}(H_2O)_5]^{2+}$-NO adduct are shown in Figure 4. The pair with the biggest eigenvalues (0.6–0.7) appears in the β-spin manifold (lower panel of Figure 4). These two channels (oriented in perpendicular planes) have an opposite character and they effectively *shift* a part of the electron density from the xz to the yz plane. This is necessary to restore a nearly cylindrical spin density in the linear Fe-N-O unit in the complex of approximate C_{4v} symmetry. Thus, rather than describing any significant inter-fragment electron transfer these two NOCV channels serve mainly to "symmetrize" the promolecular β-spin density. The other pair of NOCV channels (α-spin, upper panel of Figure 4) depict the transfer of the α-spin density from the iron core and O_{NO} to the Fe-N bonding region and yield negligible inter-fragment charge or spin transfer. In summary, no significant electron transfer between NO and the other fragment is involved while residual density delocalization from π^* on NO to the Fe-N bonding region might be anticipated, resulting in slight strengthening of the bond due to weak π covalency.

4. Conclusion

The separation of spins and proper identification of the electronic status of the fragments, "prepared" to make a bond (to minimize unnecessary spurious electron density reorganization) is indispensable to get meaningful resolution of charge and spin transfer processes and an insight into the nature and strength of the Fe-NO bond. In the case of $Fe^{II}P(NH_3)NO$ (low spin) strong π^*-backdonation may be assumed (even if somewhat opposed by σ-donation); this results in the weakening of the NO bond (experimentally evidenced by the red-shift of NO stretching frequency) and strengthening of the dative Fe-N bond [5]. On the contrary, no CT between NO and Fe fragments should be assumed in the case of $[Fe^{II}(H_2O)_5NO]^{2+}$ (high spin). Some accumulation of the electron density in the bonding region between the iron core and NO may be noticed here, thus, the formation of a weak covalent Fe-NO π bond is to be assumed also for the pentaaqua complex. This shows that the bonding nature of the Fe^{II}-NO linkage strongly depends on the environment and on the electronic state which should be covered by the relevant force constants.

Acknowledgements

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