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Hypothesis of Hemoprotein Sensor Confirmed by *ab initio* Quantum–Chemical Calculations [#]

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Abstract

The nature of the chemical bond of iron(II) porphyrin and cobalt(II) porphyrin with ligands is studied by the quantum–chemical Hartree–Fock method using the 6–31G basis set. The addition of oxygen molecule to the MeP and Im–MeP complexes (Me = Fe, Co; Im = imidazole, P = porphyrin) is established to be more favorable than water addition. It has been found that the imidazole bound to Me increases the Me–O_2 and Me–H_2O binding energies for FeP, but decreases ones for CoP. The Co atom is bound with the porphyrin ring more strongly than the Fe atom due to the larger total overlap of the atomic orbitals. The *ab initio* calculations of the complexes have demonstrated the similar changes in the structures of the geometry of the deoxyform (FeP–H_2O) of iron(II) porphyrin and the oxyform (CoP–O_2) of cobalt(II) porphyrin. This is an argument in favor of the hypothesis of hemoprotein sensor of partial oxygen tension in tissues.

Keywords. Metal porphyrins; hemoprotein sensor; ligands; cobalt ion; electronic structure.

Abbreviations and notations

Im, imidazole	P, porphyrin
His, histidine	Me, metal atom
L, ligand	HHC, hypothesis of hemoprotein sensor

1 INTRODUCTION

Cobalt, nickel and manganese ions can replace the iron ion in the porphyrinic ring [1–3] (Figure 1). The generated cobaltoheme bounds the oxygen much weaker [1] and nickel(II) porphyrin can not bound the oxygen molecule at all [2]. Gadolinium ion decreases selectively an activity of P450

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in hepatocites [4–6] and reversibly decreases the oxygen consumption by mitochondrial cytohromes ***c*** and c_1 [7].

The physico–chemical properties of cobalt(II) porphyrin and its derivatives play the most important role in regulation of functional activity of hemoproteins [1,8–14].

An injection of cobalt ions to rats leads to increasing of oxidative degradation of lever microsomal enzymes [15]. The cobalt chloride [16] leads to an increasing of concentration of glutamine in the lever and decreasing of P450–derived oxidative metabolism of acetaminophen.

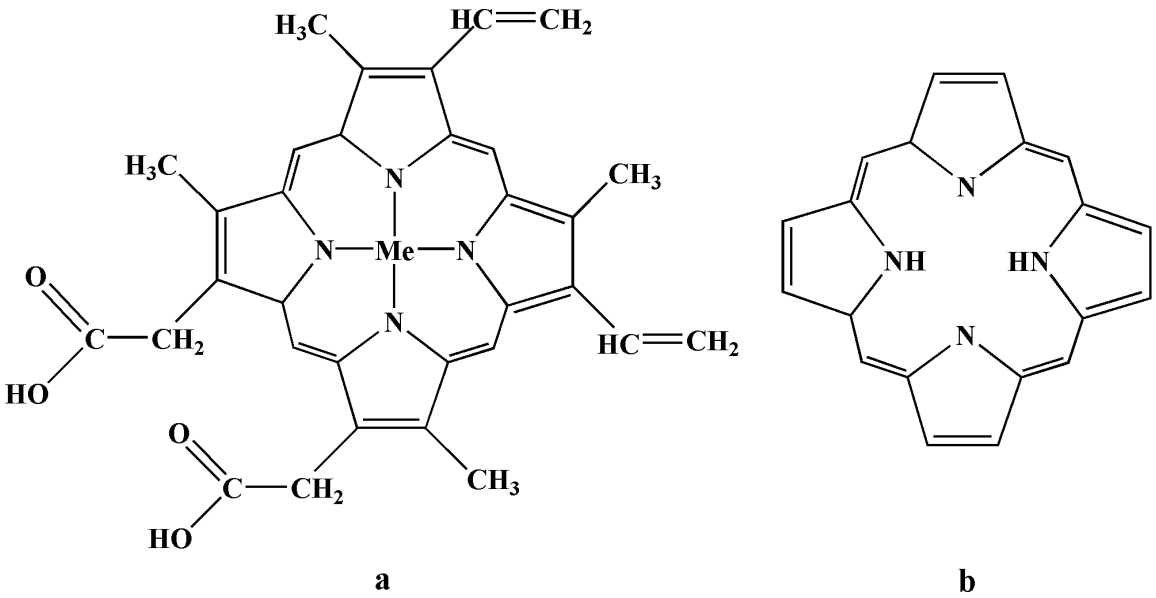

Figure 1. Molecular structures of (a) Me–protoporphyrin IX and (b) porphyrinic ring.

Cobalt ions play an exceptional role in the mechanisms of regulation of hemopoiesis under hypoxia. Introduction of cobalt into an organism in greater quantities than is necessary to its physiological needs, leads to an expression of the gene of erythropoietin, the hemoprotein factor of growth of erythroid cells [17–19].

The hypothesis of hemoprotein sensor (HHS) has been proposed in the work [20]. According to the HHS a substitution of heme iron ion by cobalt one leads to sufficient changes of heme geometry. It is well known that addition of oxygen molecule to heme structures leads to a shift of iron ion (0.4 – 0.8 Å) towards to the heme plane. These effects are followed by corresponding shift of proximal and distal histidine residues and, in more extend, affect the whole α–spiral of the protein. Thus, oxygenation of hemoglobin subunit produces a sequence of the structural changes propagated from heme to periphery [21].

The structure of the resulting deoxy– and oxy–forms of cobaltoheme is very close to the deoxy–form of hemoproteins. These changes, probably, is the main factor of inducing of expression of gene of erythropoetine. So, the main aim of the work was a verification of the HHS by using of

quantum–chemical calculations of atomic and electronic structure of MeP complexes (Me = Co(II), Fe(II), P = porphyrin) with some ligands.

Heme–containing proteins perform various functions in living organisms. In particular, they provide electron transport, reversible oxygen binding, enzymatic catalysis, etc. In hemoglobin and myoglobin molecules, the imidazole rings of two histidine residues (His) are localized on both sides of the heme plane. The nitrogen atom of proximal His forms a covalent bond with the iron atom of heme in the coordination position 5. Distal His is arranged at some distance from the heme iron atom to form a "pocket" in which ligands (O_2, NO, CO, etc.) can be incorporated.

It has been shown [22] that for electronic structure calculations of hemoglobine and mioglobine active sites it is possible to consider only heme structure. It considerably simplifies the problem and allows one to use more exact methods.

The similar approach is widely used [23–25] to study some structural subunits of biological macromolecules using semiempirical, *ab initio* and DFT methods. The electronic structure, binding energies, effective atomic charges etc. look reasonable in comparison with experimental data.

At present, it is impossible to obtain experimentally an adequate quantitative estimate of the ratio of concentrations of all substances involved in this hypothetical mechanism. However, the nature of chemical bond in iron(II) and cobalt(II) porphyrins with ligands can be studied theoretically, which allows one to compare the conformations of active sites of these molecular systems based on the HHS.

2 MATERIALS AND METHODS

To calculate the electronic structure of heme complexes with O_2 and H_2O as the first ligand in 5–th position (proximal one) with presence and absence of imidazole (Im) as the second one in distal (position No 6) one, the general atomic and molecular electronic structure system (GAMESS) [26] code has been used. Geometry optimizations searches as well as the single point electronic structure calculations for all complexes have been performed using the Hartree–Fock (HF)/6–31G level of theory.

3 RESULTS AND DISCUSSION

3.1 Comparative analysis of the energy and character of the chemical bond

The Me–O_2 and Me–H_2O binding energies (E_b) can be calculated as follows:

$$E_b = E_{\text{MeP-O}_2} - E_{\text{MeP}} - E_{\text{O}_2}, \quad (1)$$

where $E_{MeP–O_2}$ is the energy of the MeP–O_2 complex, E_{MeP} is the energy of the MeP complex, and E_{O_2} is the energy of oxygen. All calculations of the binding energy (Table 1) were carried out only for the ground states of the molecules. It has been demonstrated that the MeP and MeP–O_2 complexes have singlet ground state (the O_2 molecule has the triplet one).

A comparison of the binding energies suggests that the addition of oxygen is more favorable than water addition to the MeP and Im–MeP complexes for both the iron and cobalt atoms. The presence of Im in the 5th coordination position as the proximal ligand increases the Me–O_2 and Me–H_2O binding energies for iron by 2.5 and 1.8 times and decreases them for cobalt by factors 1.6 and 1.4.

Table 1. Metal–ligand binding energies (kcal/mol) of MeP–L complexes

Metal	The binding energies (kcal/mol)			
	Me–O_2	Me–H_2O	Im–Me–O_2	Im–Me–H_2O
Fe	–33.07	–32.00	–83.96	–59.61
Co	–83.54	–50.95	–53.40	–35.38

Note: Me–O_2 (H_2O) is a binding energy of Fe/Co ions and a ligand in the 6th coordination position, Im–Me–O_2 (H_2O) is a Fe/Co–L binding energy in the 6th coordination position with a presence of Im in the 5th one.

The difference in the binding energies (ΔE) in the CoP and FeP complexes were qualitatively estimated by the formula:

$$\Delta E = E_{FeP} - E_{CoP} - E_{Fe} - E_{Co}. \quad (2)$$

The resulting ΔE value was –37.34 kcal/mol. That indicates a stronger bond of the Co atom with the porphyrinic ring.

This result can be explained by the specific features of the metal–porphyrin chemical bond. In the FeP and CoP complexes, the formation of the bond between the metal and porphyrin is caused by overlapping of the *s*–, *p*–, and *d*–atomic orbitals of the metal and the *s*– and *p*–orbitals of the nitrogen atoms to form both σ– and π–bonds. The first ones result from an overlapping of *s*–orbitals of the metal and nitrogen atoms and Me $d_{x^2-y^2}$–orbitals with N *p* ones. The bonds of the second type are formed by overlapping of the Me d_{xz}– and Me d_{yz}–orbitals with the N p_z–orbitals.

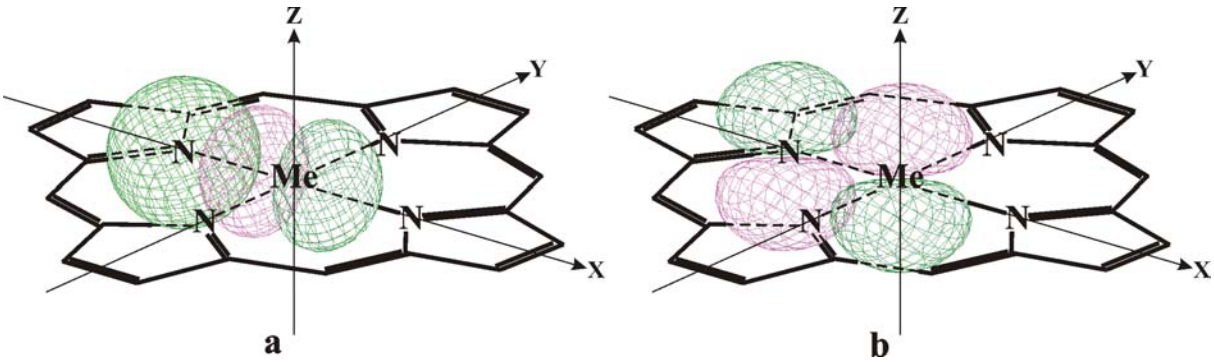

Figure 2. Overlap of the atomic orbitals: (a) *s*–orbital of the nitrogen atom of P and p_x–orbital of the Me atom; (b) p_z–orbital of the nitrogen atom of P and p_z–orbital of the Me atom.

The stronger metal–porphyrin bond in the CoP complex, unlike that in FeP, is due to the greater total overlapping of the atomic orbitals (5.377 and 5.276, respectively). The highest contribution is made by the overlaps of the *s*–, p_x–, and p_y–orbitals of the metal with the *s*–orbitals of the nitrogen atoms and by the overlaps of the p_z–orbitals of the metal and N atoms (Figure 2). The overlaps involving the *d*–orbitals of the metal atom are much smaller. The Me–N bonding orders for FeP and CoP are 0.387 and 0.575, respectively.

3.2 Comparison of the geometries of complexes

The results of our analysis of the character and degree of metal displacement for different ligands added are presented in Table 2.

Table 2. Shift (Å) of the metal atom relative to the porphyrinic ring [a]

Complexes	Shift of metal atom relative to the porphyrinic ring	Distance of the metal atom's displacement after adding the distal ligand
Im–CoP	0.176	–
Im–CoP–O_2	–0.053	0.229
Im–CoP–H_2O	0.040	0.136
Im–FeP	0.296	–
Im–FeP–O_2	0.182	0.114
Im–FeP–H_2O	0.102	0.194

[a] the negative amplitude of displacement denotes the metal atom displacement to the distal region relative to the porphyrin plane. In this case, the cobalt atom is displaced to the oxygen molecule arranged distally toward porphyrin instead of the displacement to imidazole as in all other cases.

When the ligand is in the distal position, the metal atom is shifted closer to the plane of the nitrogen atoms of the porphyrinic ring, which is more pronounced for cobalt than for iron. At the same time the results in Table 2 show that depending on the displacement of the metal atom, the cobalt complex with the water molecule imitates the iron complex with oxygen and vice versa. Hence, the (CoP–O_2) oxy form of the cobaltoheme can correspond to the geometry of the (FeP–H_2O) deoxyform of the hemoglobin heme.

4 CONCLUSIONS

The addition of the oxygen molecule to the MeP and Im–MeP complexes is more favorable than water addition for both cobalt and iron. The presence of imidazole in proximal site of the Im–MeP–O_2 and Im–MeP–H_2O complexes increases the Me–O_2 and Me–H_2O binding energies for iron and decreases them for cobalt. The cobalt atom is linked with the porphyrinic ring more strongly than iron. The geometry and electronic structure of the (FeP–H_2O) complex deoxyform are similar with to the (CoP–O_2) oxy form ones, thus supporting the hypothesis of hemoprotein sensor of partial oxygen pressure in tissues [20].

5 REFERENCES

[1] T. Yonetani, H. Yamamoto, and G. V. 3rd Woodrow, Studies on cobalt myoglobins and hemoglobins. I. Preparation and optical properties of myoglobins and hemoglobins containing cobalt proto–, meso–, and deuteroporphyrins and thermodynamic characterization of their reversible oxygenation, *J. Biol. Chem*. **1974**, *249*, 682–690.

[2] N. Shibayama, H. Morimoto and G. Miyazaki, Properties of chemically modified Ni(II)–Fe(II) hybrid hemoglobins. Ni(II) protoporphyrin IX as a model for a permanent deoxy–heme, *J. Mol. Biol*. **1986**, *192*, 323–330.

[3] M. R. Waterman and T. Yonetani, Studies on Modified Hemoglobins I. Properties of hybrid hemoglobins containing manganese protoporphyrin IX, *J. Biol. Chem*. **1970**, *245*, 5847–5852.

[4] J. Olynyk, G. Yeoh, G. Ramm, S. Clarke, P. Hall, R. Britton, B. Bacon, and T. Tracy, Gadolinium chloride suppresses hepatic oval cell proliferation in rats biliary obstruction, *Am. J. Pathol*. **1998**, *152*, 347–352.

[5] H. Yokoyama, M. Fukuda, Y. Okamura, T. Mizukami, H. Ohgo, Y. Kamegaya, S. Kato, and H. Ishii, Superoxide anion release into the hepatic sinusoid after an acute ethanol challenge and its attenuation by Kupffer cell depletion, *Alcohol Clin. Exp. Res*. **1999**, *23*, 71S–75S.

[6] A. Palaz and P. Czekaj, Toxicological and cytophysiological aspects of lanthanides action, *Acta Biochimica. Polonika* **2000**, *47*, 1107–1114.

[7] J. Ferreira, G. Tapia and L. Videla, Effects of the Kupffer cell inactivator gadolinium chloride on rat liver oxygen uptake and content of mitochondrial cytochromes, *FEBS Lett*. **1998**, *426*, 263–265.

[8] Hong–Shan He, Bai–Shan Fang, Jin–Wang Huang, Liang–Nian Ji, Catalytic studies of 2–benzthiazolethiol (BzTa)–linked manganese(III) and cobalt(II) porphyrin complexes, *Transition Metal Chemistry* **2000**, *25*, 352–357.

[9] G. E. Isom and J. L.Way, Cyanide intoxication: Protection with cobaltous chloride, *Toxicol. Appl. Pharmacol*. **1974**, *24*, 449–456.

[10] Y. Cao, J. L. Petersen and A. M. Stolzenberg, Do organocobalt porphyrins have agostic alkyl groups? An investigation of the structure of ethyl cobalt (III) octaethylporphyrin and the nuclear magnetic resonance spectra of ^{13}C–labeled alkyl cobalt (III) porphyrin complex, *Inorg. Chem. Acta* **1997**, *263*, 139–148.

[11] Chen Sh–M. and Chiu Sh–W., The catalytic and photocatalytic autoxidation of S_x^{2-} to S_4^{2-} by water–soluble cobalt porphyrin, *J. of Molecular Catalysis A: Chem*. **2001**, *166*, 243–253.

[12] H. Tang, Ch. Shen, M. Lin and A. Sen, Cobalt porphyrin–catalyzed alkane oxidation using dioxygen as oxidant, *Inorganica Chem. Acta* **2000**, *300–302*, 1109–1111.

[13] A. K. Mandal, V. Khanna, J. Iqbal, Cobalt (II) porphyrin: A versatile catalyst for the oxidation of organic substrates with dioxygen and 2–methyl propanal, *Tetrahedron Let*. **1996**, *37*, 3769–3772.

[14] D. C. Woska and B. B. Wayland, Rate constants and activation parameters for organo–cobalt porphyrin bond homolysis from NMR relaxation times, *Inorg. Chem. Acta* **1998**, *270*, 197–201.

[15] M. D. Maines and A. Kappas, Cobalt stimulation of heme degradation in the liver. Dissociation of microsomal oxidation of heme from cytochrome P–450, *J. Biol. Chem*. **1975**, *250*, 4171–4177.

[16] S. A. Roberts, V. F. Price and D. J. Jollow, The mechanisms of cobalt chloride–induced protection against acetaminophen hepatotoxicity, *Drug Metab. Disp.* **1986**, *14*, 25–33.

[17] N. Beru, J. McDonald, S. Lacomb, *et al*., Expression of the erythropoietin gene, *Mol. Cell. Biol.* **1986**, *6*, 2571–2575.

[18] S. J. Schuster, E. V. Badivas, P. Costa–Giomi, *et al*., Stimulation of erythropoietin gene transcription during hypoxia and cobalt exposure, *Blood* **1989**, *73*, 13–16.

[19] I. Beck, S. Ramirez, R. Weinmann and J. Caro, Enhancer element at the 3'–flanking region controls transcriptional response to hypoxia in the human erythropoietin, *J. Biol. Chem*. **1991**, *266*, 15563–15570.

[20] M. A. Goldberg, S. P. Dunning and H. F. Bunn, Regulation of the erythropoietin gene: evidence that the oxygen sensor is a heme protein, *Science* **1988**, *242*, 1412–1415.

[21] V. M. Stepanov, Molekulyarnaya biologiya. Struktura i funktsii belkov (Molecular Biology. Structure and Functions of Proteins), Moscow: Vysshaya Shkola, **1996**.

[22] T. A. Romanova, P. V. Krasnov and P. V. Avramov, *XVI International Winterschool on Electronic Properties of Novel Materials. Molecular Nanoclusters* **2002**, Kirchberg/Tirol, Austria, 74.

[23] C. Rovira, K. Kunc, J. Hutter, *et al*., Equilibrium Geometries and Electronic Structure of Iron–Pphyrin Complexes: A Density Functional Study, *J. Phys. Chem*. **1997**, *101*, 8914–8925.
[24] A. Dedieu, M.–M. Rohmer, M. Benard, A. Veillard, Oxygen binding to iron Porphyrins. An *ab initio* calculation, *J. Am. Chem. Soc*. **1976**, *98*, 3717–3718.
[25] C. Rovira, P. Carloni, M. Parrinello, The Iron–Sulfur Bond in Cytochrome *c*, *J. Phys. Chem. B* **1999**, *103*, 7031–7035.
[26] M. W. Schmidt, K. K. Baldridge, J. A. Boatz, *et al*., General atomic and molecular electronic structure system, *J. Comp. Chem*. **1993**, *14*, 1347–1363.

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