April 23, 1997

Spin Transitions in Heme Complexes as Related to CO and O_2 Myoglobin Binding Kinetics

A.L. Tchougréeff
Karpov Institute of Physical Chemistry,
Vorontsovo pole 10, Moscow 103064, Russia

We present results of the ECF study on the spin and symmetry of the ground and lower excited states of deoxymyoglobine modelled by imidazolato iron(II) porphyrine for a range of molecular geometries. It is found that for deformations of the heme structure which preserve the distance between the iron atom and the imidazole N_ε-atom a spin transition takes place from the quintet state (which is the ground state for the experimental geometry of the heme where the distance between the iron atom and porphyrine ring center is about 0.5 Å) to the singlet state which becomes a ground state for the distances between the iron atom and the ring center shorter than 0.4 Å. In the vicinity of the transition point an intermediate spin triplet state has the energy in a range of magnitude characteristic for the spin-orbit coupling constant of iron(II) ions in the porphyrin complexes. On the other hand a relatively small (by ~ 0.15 Å) elongation of the Fe-N_ε bond length results in a transition to the triplet (^3E_g) ground state. Relation of the found system of the heme electronic states to the features of the CO and O_2 binding kinetics is discussed.

The kinetics of binding of small molecules like CO, O_2, or NO by myoglobine (Mb) remains a source of some controversy. Experimental data on CO and O_2 binding kinetics at ambient pressure (for review see [1]) demonstrate unusual similarity of the corresponding reaction kinetics parameters (activation enthalpies and preexponential factors). This feature is particularly difficult to explain since these two molecules have different total spin in their respective ground states which according to [1] had to result in significant distinctions in their reactivity towards the quintet ground state of Mb. In papers [1-3] some theoretical explanations for this unexpected similarity have been proposed. Some of them relate this behaviour to diffusive character of the rate determining step. However, it is in contradiction with the effect of external pressure on the binding kinetics by Mb which has opposite sign for CO and O_2 : applying pressure increases the rate of CO binding and decreases that of O_2 [1]. This is not consistent with a purely diffusive character of the kinetics.

Let us note that whatever binding kinetics is in nature it is ultimately controlled by the electronic states of its reactant and product molecules. The CO and O_2 molecules have respectively singlet ($^1\Sigma^+$) and triplet ($^3\Sigma_g$) ground states. Both adducts of either CO or O_2 with myoglobine (MbCO or MbO_2) have singlet ground states and deoxyMb (iron(II) porphyrin with an axial imidazole ligand) has a quintet (high spin, HS) ground state. According to the well known spin coupling rules the binding of both CO and O_2 is a spin forbidden process for the ground (HS) state of the heme since a change of the total spin of either systems (Mb + CO or Mb + O_2) is required in some point along a reaction path of the binding. However, the details of the spin-orbit interaction are different and namely this difference made the authors [1] to expect that the binding kinetics for these two molecules has to be different in contradiction with experiment. It is known, however [4,5], that there is a number of electronic states of the heme (iron(II) porphyrine) in a narrow low-energy segment which differ by their total spin and spatial symmetry and each of them can become a ground state depending on subtle details of the geometry of the close ligand sphere of the iron ion. For example, iron(II) porphyrin (four coordinate) has a triplet (^3E_g, intermediate spin) ground state, six coordinated iron(II) porphyrins have singlet (low spin, LS) ground state, and the five-coordinate iron (II) porphyrine (like that in Mb) has quintet ground state (see above). Already in [6] it was proposed that a spin transition to some "lower spin" state unspecified in [6] makes the heme reactive towards dioxygene. (Clearly, for the reaction of the triplet dioxygen molecule with iron(II) porphyrin to become spin allowed it is necessary that the iron porphyrin is in a triplet state as well). Moreover, it was also mentioned in [6] that the change of two distances, namely, of the iron shift perpendicular to the porphyrine plane and of the distance between the iron atom and imidazole nitrogen atom might be of importance for the small molecule binding to the heme.

In the present paper we studied dependence of the ground state spin and symmetry of the iron(II) porphyrine imidazole complex on some details of the ligand geometry around the metal ion. Following suggestions of paper [6] we considered a two dimensional slice of the coordinate space spanned by two coordinates: that of the iron shift perpendicular to the porphyrine plane r_{Fe-Ctr} and the distance between the iron atom and imidazole nitrogen atom r_{Fe-N_ϵ}, and calculated the ground and several low-energy excited states of the complex for a grid points in this coordinate area. Additional question we addressed and which was of interest as well was that concerning the relative energy of the intermediate spin (triplet) state relative to the HS and LS ones. According to the general theory of LS $\rightarrow$ HS transitions in the iron(II) complexes [7] proximity of this state is important for effective second order spin-orbit coupling between the HS and LS states ultimately responsible for the transition.

Electronic structure of metal porphyrins and of iron porphyrins, in particular, is an important problem for quantum chemistry. It was addressed both on *ab-initio* and semi-empirical levels of the theory. Unfortunately, the results of their application were not particularly advantageous. Indeed, even for the simplest iron(II) porphyrin some doubts [4,5] still persist concering the ground state spin and symmetry. Moreover, the *ab-initio* approaches are too expensive to use them for screening of large portions of the nuclear configuration space. In order to perform this we applied the effective crystal field (ECF) method, proposed recently [8] for description of low-energy states of transition metal complexes. This method has been implemented and extensively tested (see [9] and references therein) on a series of the complexes of the first transition metal row. In the context of the present study the most important test was its success in the case of metal porphyrins [9] and of the spin transition in the *cis*-[Fe(bipy)$_2$(NCS)$_2$] complex [10]. It turned out to be possible to reproduce the change of the total spin of this quasioctahedral d^6 complex from the LS to HS form when the Fe-N distance increases by $\sim 0.15 \div 0.20$ Å in agreement with experiment [11]. It was also possible to describe several low-energy d-d excited states for the both spin states in acceptable agreement with experiment [12].

The results of our calculations are depicted on Fig. 1. One can see that in a rectangle with $r_{Fe-Ctr} = 0.0 \div 0.5$ Å and $r_{Fe-N_\epsilon} = 2.0 \div 3.0$ Å three types of the ground state with three respective values of the total spin are possible. Within the ECF/CNDO approach [8-10] the ground state is the singlet (LS) $^1A_{1g}$ state in a narrow area along the $r_{Fe-N_\epsilon} = 2.0$ Å axis if only the r_{Fe-Ctr} distance is smaller than 0.45 Å. Between $r_{Fe-Ctr} = 0.4$ Å and $r_{Fe-Ctr} = 0.45$ Å a spin transition to the HS $^5B_{2g}$ state occurs. The latter state is the ground state only in a small region in the right lower corner of the coordinate area we consider. The experimental geometry of deoxymyoglobin falls namely in this area, and according to [4,5] Mb has an HS ground state. By contrast, even relatively small elongation of the Fe-N$_\epsilon$ bond results in a spin transition to the triplet (intermediate) ^3E_g ground state. It is not very surprising since the ECF/CNDO calculation yields this ground state for the four coordinated iron(II) porphyrine (without any axial ligand) [9] in agreement with experiment [13]. Somewhat unexpected is the fact that a relatively small elongation of the Fe-N$_\epsilon$ bond (by $0.1 \div 0.15$ Å) results in the spin transition to the triplet state whereas the deformation required to reach the quintet state is much larger.

One can see, that in the vicinity of the LS $\rightarrow$ HS transition point the intermediate spin (triplet) state has very low energy. For example, at $r_{Fe-Ctr} = 0.4$ Å (before the transition, $^1A_{1g}$ ground state) the energy of the excited $^5B_{2g}$ state amounts 877 cm^{-1} and that of the excited ^3E_g state is 437 cm^{-1}. At the distance $r_{Fe-Ctr} = 0.45$ Å (after the transition) the states $^1A_{1g}$ and $^5B_{2g}$ change their relative position (the latter becomes the ground state and the former - excited one), however, the energy of the intermediate ^3E_g state remains pretty close to the both: it equals to 874 cm^{-1}, whereas the energy of the singlet is 626 cm^{-1}. In addition one more quintet state intrudes in the same energy range: the ^5E_g state has the energy 665 cm^{-1} above the HS ground state. It is clear that these energy differences are comparable with the spin-orbit interaction which constant ζ is about 400 cm^{-1} in the iron complexes [7]. For that reason in the vicinity of the transition point the ground state is not a state with some definite total spin (whatever appears from the ECF calculation without spin-orbital interaction) but contains significant contributions from all states with the total spin values 0, 1, and 2. The energy profile along the coordinate consisting of simultaneous shift of the iron atom and imidazole ligand (with the conserved distance $r_{Fe-N_\epsilon} =$ 2.0 Å) is consequently more likely adiabatic rather than nonadiabatic [1]. At the same time for the geometries close to the respective minima on the potential energy surfaces the values of the total spin of the corresponding ground and excited states are well defined since the energy differences are large as compared to the spin-orbital interaction constant ζ. For example, at the deoxyheme HS minimum which is believed to lay at $r_{Fe-Ctr} = 0.5$ Å; $r_{Fe-N_\epsilon} = 2.0$ Å [1,4] the excitation energies are as follows: $\Delta E(^5B_{2g} \to {}^5E_g) = 934$ cm^{-1}; $\Delta E(^5B_{2g} \to {}^1A_{1g}) = 2310$ cm^{-1}; and $\Delta E(^5B_{2g} \to {}^3E_g) =$ 2087 cm^{-1}. At the LS minimum $r_{Fe-Ctr} = 0.0$ Å; $r_{Fe-N_\epsilon} = 2.0$ Å respective energies are as follows:$\Delta E(^1A_{1g} \to {}^5E_g) = 2878$ cm^{-1}; $\Delta E(^1A_{1g} \to {}^3B_{2g}) =$ 5356 cm^{-1}; and $\Delta E(^1A_{1g} \to {}^5E_g) = 7793$ cm^{-1}.

In conclusion let us speculate about the puzzling phenomenon of similarity of the binding kinetics of CO and O_2 to myoglobine [1]. On the basis of the difference of the ground state spin of CO (singlet) and O_2 (triplet) authors [1] expected significant distinction in their respective Mb binding kinetics. Since the total spin of the both products is the same (singlet), thus a different degree of the spin change in the course of the binding of each small molecule by the heme is necessary [1]. Surprizingly, the parameters of the overall kinetics are essentially the same for the both small molecules [1]. The explanations of this contradiction in the literature [1-3] are based on the assumption on the diffusive character of the binding kinetics, which in its turn contradicts to the observed pressure effects on the binding kinetics (see above). Our proposition is to leave up considering the coordinate related to approach of a small molecule to the heme, but to consider a multidimensional configuration space (in our case - the two-dimensional one) of the heme itself. This proposition is based on the accepted idea [6] that the slow motions of the protein backbone being transferred to the iron ion can change its spin state and thus its reactivity towards dioxygen binding. The motions of the axial imidazole ligand may bring the heme unit to areas in the coordinate space where it becomes either singlet or triplet. Since the backbone motions are slow there is enough time for spin transitions to either state. For the respective states of the heme the binding of CO and O_2 are spin allowed processes and the measured energy barriers appear due to some steric interactions which may be similar for the similar small molecules. The overall binding kinetics of respective small molecules is is determined also by the rate with which the heme enters the areas in the coordinate space where it has the ground state of the total spin required for either reaction. Since the protein backbone motions are slow there is enough time for transitions to any spin state of the heme. This proposition agrees with the available data on electronic structure of the respective adducts. For MbCO it can be presented as $^1[\mathrm{Mb}(t_{2g}^6\ {}^1A_1)\mathrm{CO}(^1\Sigma^+)]$ (Ref. 1), whereas for the $\mathrm{MbO_2}$ the electronic structure is different $^1[\mathrm{Mb}(t_{2g}^5 e_g\ {}^3E_g)\mathrm{O_2}(^3\Sigma_g)]$ (Ref. 14). These electronic states correlate with the states of Mb we considered as reactive with respect to the corresponding binding. This proposition agrees also with the observed effects of pressure on the kinetics of the CO and O_2 binding. One can expect that increasing pressure would restrict elongation of the Fe-N_ϵ bond and by this would hinder the access of the system to the area of triplet ground state where the reaction with O_2 is spin allowed. At the same time the area of the singlet ground state where the CO rebinding is spin allowed becomes more accessible. This gives a plausible explanation to the experimentally observed pressure effects.

Acknowledgments.

The author acknowledge fruitful discussion with Dr. A.M. Berezhkowskii and G.V. Davidovich who attracted his attention to the problem of electronic states of myoglobin. The author is thankful to Dr. A.V. Soudackov for his kind help in the data praparation and to I.A. Misurkin for his kind comments concerning the style. This work is performed with financial support from the INTAS through the grant 94-4089.

References.

[1] H. Frauenfelder, P.G. Wolynes. Science. 229 (1985) 337.

[2] N. Agmon, J.J. Hopfield. J. Chem. Phys. 79 (1983) 2048; *ibid.* 80 (1984) 592.

[3] J. Jortner, J. Ulsturp. J. Am. Chem. Soc. 101 (1979) 3744.

[4] A. Dedieu, M.-M. Rohmer, A Veillard. Adv. Quant. Chem. 16 (1982) 43.

[5] D.C. Rawlings, M. Gouterman, E.R. Davidson, D. Feller. Int. J. Quant. Chem. 28 (1985) 773.

[6] M.F. Perutz. Nature 228 (1970) 734; *ibid.* 237 (1972) 495.

[7] E. König. Struct. & Bonding. 76 (1991) 53.

[8] A.V. Soudackov, A.L. Tchougréeff, I.A. Misurkin. Theor. Chem. Acta. 83 (1992) 389.

[9] A.V. Soudackov, A.L. Tchougréeff, I.A. Misurkin. Int. J. Quant. Chem. 58 (1996) 161.

[10] A.L. Tchougréeff, A.V. Soudackov, I.A. Misurkin, H. Bolvin, O. Kahn. Chem. Phys. 193 (1995) 19.

[11] B. Gallois, J.-A. Real, C. Hauw, J. Zarembowitch. Inorg. Chem. 29 (1990) 1152.

[12] P. Gütlich. Struct. Bonding. 44 (1981) 85.

[13] T. Kitagawa, J. Teraoka. Chem. Phys. Lett. 63 (1979) 443.

[14] B.D. Olafson, W.A. Goddard. Proc. Natl. Acad. Sci. U.S.A. 74 (1977) 1315.

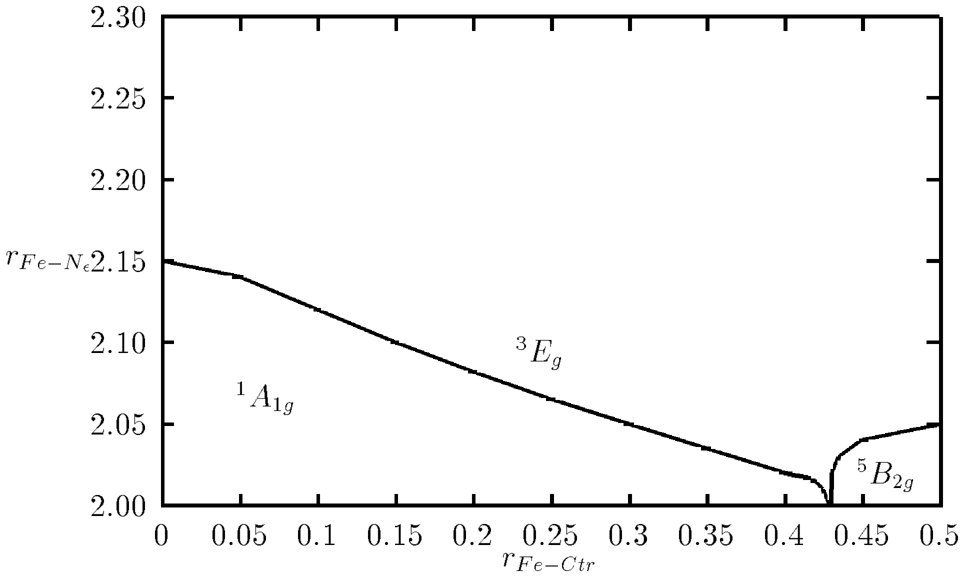

Figure 1: The map of the coordinate space slice with the areas of respective ground state spin of the heme