Mössbauer Spectroscopy as a Probe of Electric Field in Heme Pocket of Deoxyheme Proteins: Theoretical Approach

Alexandr Gorski[1], Aleksandr Starukhin[2], and Solomon S. Stavrov[3,*]

[1]Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka, 44/52, 01-224 Warsaw, Poland,
[2]B.I. Stepanov Institute of Physics, National Academy of Science of Belarus, Nezalezhnasti Ave., 70, 220072 Minsk, Belarus
[3]Sackler Institute of Molecular Medicine, Department of Human Molecular Genetics and Biochemistry, Sackler School of Medicine, Tel Aviv University, Tel Aviv 69978, Israel.

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Alexandr Gorski[1], Aleksandr Starukhin[2], and Solomon S. Stavrov[3,*]

[1]*Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka, 44/52, 01-224 Warsaw, Poland,*

[2]*B.I. Stepanov Institute of Physics, National Academy of Science of Belarus, Nezalezhnasti Ave., 70, 220072 Minsk, Belarus*

[3]*Sackler Institute of Molecular Medicine, Department of Human Molecular Genetics and Biochemistry, Sackler School of Medicine, Tel Aviv University, Tel Aviv 69978, Israel.*

Abstract

Chemical reactions taking place in active centers of different enzymes are controlled by electric fields created by the protein in these centers. These electric fields can be experimentally detected by different experimental techniques (infrared absorption, NMR, *etc.*). In this paper, we use quantum chemical calculations to show that Mössbauer spectroscopy can be also used to study protein electric field. We study effect of both the model and protein electric fields on the magnitude of quadrupole splitting of Mössbauer spectra of the high-spin ferrous myoglobin and its models. It is shown that the quadrupole splitting is notably affected by the protein electric field. This result also explains a number of the experimental data.

Keywords

heme proteins; myoglobin; hemoglobin; protein dynamics; porphyrin.

Introduction

.Relationship between structure, dynamics and function of enzymes is one of the major problems of modern biochemistry and biophysics. It is clear that environment of the active center of an enzyme creates an electric field (EF), the latter affecting chemical reactions taking place in the active center (see, for example [1]). Therefore, it is very important to study this EF and its spatial distribution.

Heme proteins (HPs) are widely used to address this problem, because they can be studied by optical and infrared absorption, NMR, Raman scattering and many other experimental techniques. The effect of the heme environment on the electronic structure, spectra and properties of the heme active center have been studied both experimentally (for reviews see [2-7]) and theoretically using the vibronic theory of activation [8-13] and direct quantum chemical calculations.[14-18,3,19,20] It was shown that in carbon monoxide complex of myoglobin (MbCO) the electronic structure and, consequently, the spectra (^{13}C, and ^{17}O nuclear magnetic resonance spectra, optical absorption and infrared absorption spectra) are notably affected by the heme environment EF. Comparison of the experimentally observed C-O vibrational frequency, ν(CO), and the dissociation rate constants of CO, NO and O_2 of different Mb mutants with the calculated EF in the heme pocket showed [4] that the protein EF affects both the ν(CO) and the affinity of the heme for these diatomic ligands. A recent study of the CO complex of horseradish peroxidase showed that not only the position of the CO infrared band, but also its width is very revealing, providing specific information on the dynamics of the heme environment (see, for example [21-24]).

The quadrupole splitting (ΔE_Q) of the excited nuclear state of iron isotope ^{57}Fe is observed in Mössbauer spectra [25-32] and provides one with direct information about the inhomogeneity of the electric field (electric field gradient, EFG), produced on the iron nucleus by its environment, both the electron cloud and external electric field. ΔE_Qs of HPs and iron porphyrin complexes were extensively studied theoretically.[15,16,33,34] These studies showed that application of the DFT approach produces good results on computations of ΔE_Qs.

Effect of the protein EF on ΔE_Q of only closed-shell HPs with big energy gap between the excited states and the ground one was studied earlier; it was shown to be very weak.[16] This result is well understood, because admixture of the excited states to the ground one by external perturbations is weak and, consequently, distribution of electronic cloud around the iron nucleus in such compounds is weakly affected by the perturbations.

At the same time in HPs containing open-shell iron its electronic structure is expected to be much more sensitive to any perturbations (including EF) than that of closed-shell heme proteins, because the energy gap under consideration is much smaller. However, to our best knowledge effect of external EFs on ΔE_Q of these compounds was not studied theoretically.

In this letter we report results of theoretical study of effects of different model EFs on ΔE_Q of the high-spin (S=2) iron-porphin-imidazole complex (Fe(P)(Im)), model of the Mb active center, and of EF of the distal environment of the heme on ΔE_Q of myoglobin.

Theory

ΔE_Q of the iron atom is controlled by the principal components of the EF gradient tensor (V_{zz}, V_{yy}, and V_{xx}, $|V_{zz}| > |V_{yy}| > |V_{xx}|$) on this atom.

$$\Delta E_Q = \frac{1}{2} e Q V_{zz} \left(1 + \frac{\eta^2}{3}\right)^{\frac{1}{2}}, \tag{1}$$

where

$$\eta = \frac{V_{xx} - V_{yy}}{V_{zz}}, \tag{2}$$

e is the electron charge and $Q = 0.16 \cdot 10^{-28} \cdot m^2$ is quadrupole moment of the ^{57}Fe I* = 3/2 excited state.[15]

To check how different model EFs and the EF of the closest heme environment affect ΔE_Q, we computed V_{zz}, V_{yy}, and V_{xx} using the DFT approach utilizing pure functional BPW91 (Becke 88 exchange and PW91 correlation functionals); spin unrestricted method; and Wachter's all electron basis set for iron, 6-311G* set for other heavy atoms, and 6-31G* set for hydrogen atoms [15], as it was implemented in the Gaussian 03 package [35]. Note, that this approach was shown to reliably calculate ΔE_Q of isolated active centers of different closed- and open-shell HPs.[15]

Results and Discussion

In the beginning, the geometry optimization of the high-spin Fe(P)(Im) complex was performed. Then the electronic structures of Fe(P)(Im) in the presence of E = 0.01 a.u. homogeneous EFs (1 a.u. = $5.14 \cdot 10^9$ V/cm , this magnitude of the field being of the order of the field in the heme pocket [4,36]) directed parallel ($E^{\parallel}$) and perpendicular ($E^{\perp}$) to the porphyrin plane, were calculated. Effects of changes in the iron out of the porphyrin plane displacement (r) and distance between the iron and nitrogen of the proximal imidazole (R) were also studied.

The geometry optimization of the high-spin Fe(P)(Im) complex yielded r = 0.32 Å and R = 2.12 Å.

To understand how strong the EF effect is, one has to compare this effect to the one of the heme distortions. To do that we calculated the effect of two widely discussed and functionally important heme distortions: changes in r and R to 0.42 Å (note that in hemoglobin r ≈ 0.4 Å [37]) and 2.27 Å, respectively. Note, that elongation of the covalent iron-imidazole bond R by 0.15 Å substantially affects the electron cloud around the iron atom.

The results are presented in Table 1. They show that both the distortions and EFs affect ΔE_Q, the perpendicular to the porphyrin plane EF affecting ΔE_Q not less, than the distortions under consideration. The field parallel to the porphyrin plane hardly changes ΔE_Q. Note, that homogeneous EF by itself does not affect ΔE_Q. Consequently, the change in ΔE_Q reflects the reorganization of electron cloud around the iron nucleus caused by the homogeneous EF.

Table 1 Influence of the distortions and of the homogeneous EF on ΔE_Q of the high-spin Fe(P)(Im)

	ΔE_Q mm/s	η
r = 0.32 Å, R = 2.12 Å E = 0	-2.06	0.77
r = 0.42 Å, R = 2.12 Å E = 0	-2.13	0.97
r = 0.32 Å, R= 2.27 Å E = 0	-2.18	0.48
r = 0.32 Å, R = 2.12 Å $E^{\parallel}$ = 0.01 a. u.	-2.06	0.76
r = 0.32 Å, R = 2.12 Å $E^{\perp}$ = 0.01 a. u.	-1.94	0.91
r = 0.32 Å, R = 2.12 Å, $E^{\perp}$ = -0.01 a. u.	-2.11	0.64

The effect of the closest heme environment on ΔE_Q of Mb was also computed. To do this we used the X-ray data [38]. We simulated the distal and proximal histidines with imidazoles and neglected the contribution of the peripheral porphyrin residues. The latter assumption is based on (a) the fact that these residues do not participate in the porphyrin pi-conjugation, and (b) our finding that EF parallel to the porphyrin plane hardly affects ΔE_Q, see Table 1. The relative positions of heavy atoms of the

heme, distal imidazole, and hydrogen bonded water molecule were taken from [38], then the hydrogen atoms were added and their positions were refined by the geometry optimization using the same quantum chemical approach, see Fig. 1.

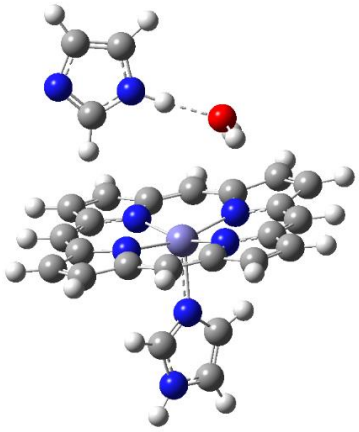

Fig. 1 Structure of heme and its distal environment in myoglobin [38] with optimized positions of the hydrogen atoms (Fe – violet, O – red, N – blue, C – grey, H – white)

Using this structure, we computed ΔE_Q of the heme-imidazole complex with and without the distal environment, the results being presented in Table 2.

Table 2 Influence of EF of the distal imidazole and the water molecule on ΔE_Q of myoglobin heme

	ΔE_Q, mm/s	η
Heme	-2.08	0.68
Heme and distal environment	-2.18	0.69

It follows from Table 2 that EF of the distal environment, histidine and water changes ΔE_Q by 0.1 mm/s, this change being just a beat weaker, than the effect of the elongation of the covalent iron-imidazole bond by 0.15 Å, 0.12 mm/s, see Table 1. It follows from this comparison, that the protein EF can affect ΔE_Q of deoxyheme proteins to the same extent as relatively large heme structural changes. Consequently, the protein EF effect is notable and measurable and has to be taken into account when interpreting Mossbauer spectra of these proteins.

Note, that taking into account the EF of the heme environment improves the theoretical result, making it considerably closer to the experimentally observed value of -2.22 mm/s [39], but hardly affects η.

It follows from the results presented above that both the model and protein electric fields notably affect the quadrupole splitting of the ferrous iron of Fe(P)(Im) and of ferrous deoxyheme proteins.

To our best knowledge, the results presented in this letter are the first demonstration of the effect of an external EF on ΔE_Q. It stems from the re-organization of the electronic cloud around the iron nucleus caused by the EF.

This effect of the protein EF can explain, at least partially, the deviation between the experimental results and those, obtained without taking into account the EF.[15] It also can contribute to the broadening of the deoxyhemoglobin Mössbauer spectra [30] as a result of different heme environment in α and β subunits of hemoglobin.[37]

Conclusion

It follows from the results presented above that external electric field can notably affect quadrupole splitting of complexes with open-shell metal atoms. As such, on the one hand, it must be taken into account when interpreting the experimental data. On the other hand, it can be used as an effective probe of external electric fields, including protein electric field.

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