

The Effect of Iron Displacement Out of the Porphyrin Plane on the Resonance Raman Spectra of Heme Proteins and Iron Porphyrins

Solomon S. Stavrov

Sackler Institute of Molecular Medicine, Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Tel Aviv 69978, Israel

ABSTRACT The causes of the strong coupling of the iron-histidine vibration to the Soret resonance in the resonance Raman spectra of deoxyhemoglobin, myoglobin, and peroxidase are explored, using the vibronic theory. It is shown that the extent of the iron displacement out of the plane of the porphyrin nitrogens is the main structural parameter controlling the Fe-N_{His} band features, such as the dependence of its frequency and intensity on the protein conformation and number of the axial ligands, time evolution after the photolysis of the diatomic complexes of the proteins under consideration, and inverse relationship between the changes Fe-N_{His} and ν_4 porphyrin breathing mode frequencies.

1. INTRODUCTION

The mechanism of the interaction between the active center and the protein globule in the heme proteins was subject to numerous investigations (see, for example, Gelin et al. (1983), Frauenfelder et al. (1988), Rousseau and Friedman (1988), Perutz (1989)). In hemoglobin (Hb), myoglobin (Mb), and peroxidase (PO), the bond between iron and the proximal histidine imidazole nitrogen, Fe-N_{His}, is the only covalent bond between the heme and the globule (Frauenfelder et al., 1988; Kitagawa, 1988; Rousseau and Friedman, 1988; Perutz, 1989). Therefore, any experimental technique which allows to study this bond directly, is very useful for the elucidation of the interaction under consideration.

It was previously shown (Hori and Kitagawa, 1980; Terakawa and Kitagawa, 1981; Collman et al., 1983; Kitagawa, 1988; Rousseau and Friedman, 1988), that the resonance Raman scattering spectra of Hb, Mb, and PO and their model compounds contain bands in the region of 200–250 cm⁻¹ which correspond to the Fe-N_{His} vibration. The frequencies and intensities of these bands essentially depend on the nature and conformation of the protein, temperature and kind of solvent (Kitagawa, 1988; Rousseau and Friedman, 1988). It was also shown that in the heme proteins the Fe-N_{His} frequency, $\nu(\text{Fe-N}_{\text{His}})$, inversely correlates to the frequency of the porphyrin vibration, ν_4 , which is mainly the pyrrole C—N vibration. Both bands were widely used to study the dependence of the structure and conformation of the heme proteins on the temperature, allosteric effectors, etc. Particularly useful was the study of these bands in the investigation of the dynamics of the proteins in combination with the flash-photolysis technique (Rousseau and Friedman, 1988).

It was proposed (Friedman et al., 1982) that the coordination geometry (see Fig. 1) of the histidine imidazole (the extent and direction of its tilt) is the main parameter of the compound that regulates the position and intensity of the Fe-N_{His} band. This point of view was supported by the authors of the resonance Raman study of Mb and Hb (Bangchaoenpaupong et al., 1984), where the Fe-N_{His} vibration was shown to be coupled directly to the Soret excitation, which corresponds to the porphyrin ring $\pi \rightarrow \pi^*$ excitation. Such a result was not trivial, because the nature of the Fe-N_{His} bond (strength, interatomic distance, force constant, etc.) is controlled mainly by the nature and population of the $\sigma(\text{Fe-N}_{\text{His}})$ iron-imidazole orbital. The latter is an antibonding component of the overlapping d_{z^2} iron and p_z imidazole nitrogen orbitals, and it is elongated in the axial direction, perpendicular to the porphyrin plane. Therefore marked intensity of the Fe-N_{His} band in the resonance Raman spectrum implies that the porphyrin and axial electronic subsystems are not orthogonal in the compounds under consideration. To explain this experimental result it was proposed (Bangchaoenpaupong et al., 1984) that the tilt of the proximal imidazole reduces the D_{4h} symmetry of the heme, strongly mixing the $\sigma(\text{Fe-N}_{\text{His}})$ and $e_g(\pi^*)$ molecular orbitals (MOs), and the extent of this mixing essentially depends on the direction of the tilt. This assumption, called below as BSC model, allowed to explain qualitatively a number of experimental data (see Section 3).

At the same time it was mentioned (Champion, 1988) that "a mixing of the $a_{1u}, a_{2u} \rightarrow e_g$ porphyrin orbital transitions with spin allowed excitations involving the d_{z^2} iron orbital" can lead to the coupling of the Fe-N_{His} vibration to Soret resonance. Such a mixing can take place due to the reduction of the D_{4h} symmetry of the heme to C_{4v} upon displacement of the iron out of the plane of the porphyrin nitrogens even if the axial symmetry is conserved. In this paper we will discuss in detail the influence of this displacement on the nature of the ground and excited states of heme proteins and their models, position, and intensity of the Fe-N_{His} and ν_4 bands and reanalyze the pool of experimental data on the structure, conformation, and dynamics of the heme proteins

Received for publication 28 December 1992 and in final form 15 July 1993.

Address reprint requests to Solomon S. Stavrov at the Sackler Institute of Molecular Medicine, Sackler Faculty of Medicine, P.O.B. 39040, Tel Aviv University, Ramat Aviv, Tel Aviv 69978, Israel. Tel.: 972-3-640-9859 (lab); 972-3-579-9876 (home); Fax: 972-3-641-4245; e-mail: stavrov@ccsg.tau.ac.il.

© 1993 by the Biophysical Society

0006-3495/93/11/1942/09 \$2.00

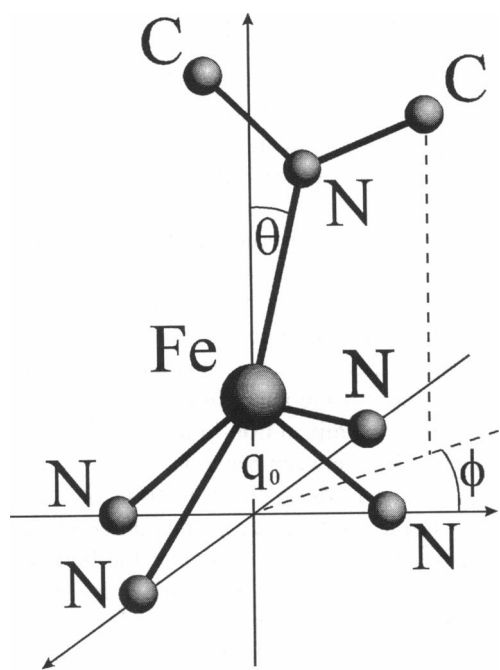


FIGURE 1 Schematic representation of the structure of the heme-imidazole unit.

from this point of view (preliminary results of this analysis were presented by Stavrov (1992)).

2. THE ORIGIN OF THE IRON OUT-OF-PLANE DISPLACEMENT IN THE FIVE-COORDINATED METALPORPHYRINS AND THEIR ELECTRONIC STRUCTURE

In our previous works (Bersuker et al., 1979; Bersuker and Stavrov, 1981, 1983, 1988; Stavrov and Bersuker, 1983) the electronic causes for the iron displacement out of the plane of the porphyrin nitrogens, q , in the heme proteins and metalloporphyrins, Me(P), were investigated. To do this we studied the simultaneous effect of the axial-ligand "crystal field," of A_{2u} symmetry, and of the electron-nuclear (vibronic) interaction with the q displacement of the same A_{2u} symmetry (here and below we use notations of the D_{4h} point symmetry group). For complexes with one axial ligand the second order perturbation theory in the one-electron approximation led to the following expressions for the valence MOs ψ_i

$$\psi_i(q) = \psi_i^{(0)}(0) - \sum_j \frac{v_{ij}}{2\delta_{ji}} \psi_j^{(0)}(0) - q \sum_j \frac{f_{ij} v_{ij}}{2\delta_{ji}} \psi_j^{(0)}(0) \quad (1)$$

and their energies ϵ_i

$$\epsilon_i(q) = \epsilon_i(0) - \Delta_i - q \sum_j \frac{f_{ij} v_{ij}}{\delta_{ji}} - q^2 \sum_j \frac{f_{ij}^2}{2\delta_{ji}} \quad (2)$$

where $\psi_i(0)$ is the i th MO of the pure imidazole or Fe(P), calculated for the iron-in-plane nuclear configuration of the latter ($q = 0$); $\epsilon_i(0)$ is its energy; Δ_i , a term that de-

scribes the change in MO energy due to complex formation, and does not depend on q ; $2\delta_{ij} = \epsilon_i(0) - \epsilon_j(0)$ is an energy gap between the i th and j th MOs, and v_{ij} and f_{ij} are the corresponding one-electron matrix elements of the ligand "crystal field" and vibronic operators.

We can obtain an expression for the whole electronic energy of the system by summing over the valence MO energies, multiplied by their electronic population numbers, p_i , and adding the term $\frac{1}{2}K_0q^2$, that describes the elastic energy of inner orbitals

$$E = E_0 - \Delta - q \sum_{i,j} p_i \frac{f_{ij} v_{ij}}{\delta_{ji}} + \frac{1}{2} q^2 \left(K_0 - \sum_{i,j} p_i \frac{f_{ij}^2}{\delta_{ji}} \right) \quad (3)$$

where E_0 is a whole energy of the pure Fe(P) and imidazole and Δ describes the energy gain due to the complex formation and does not depend on q . Taking advantage of Eq. 3 we can calculate a position of the energy minimum of the system in the q direction, which corresponds to the electron-vibronic contribution into the iron out-of-plane displacement q_{ev} :

$$q_{ev} = \left(\sum_{i,j} p_i \frac{f_{ij} v_{ij}}{\delta_{ji}} \right) \left(K_0 - \sum_{i,j} p_i \frac{f_{ij}^2}{\delta_{ji}} \right)^{-1} \quad (4)$$

We must notice that the contributions of the mixing MOs with the same population numbers to the sums of Eqs 3 and 4 cancel each other. Therefore, the mixing of the iron porphyrin MOs with different p_i only contributes to the dependence of the whole electronic energy on q , leading to the iron out-of-plane displacement in the complexes with one axial ligand.

It was shown (Bersuker et al., 1979; Bersuker and Stavrov, 1981, 1983, 1988; Stavrov and Bersuker, 1983), that the main contribution to q_{ev} results from the mixing of the $a_{1g}(d_{z^2})$ and $a_{2u}(\pi)$ orbitals, the population of the $b_{1g}(d_{x^2-y^2})$ metal orbital being also important for the reduction of the K_0 value. Using this conclusion we can estimate q_{ev} value. The resulting force constant can be calculated from the experimental data on the mean-square amplitude of the iron atom at different temperatures (Parak et al., 1982). This amplitude was attributed to the low-frequency iron out-of-plane movement (Li and Zgierski, 1992), and its force field constant can be calculated in the high-temperature limit from the relationship $\frac{1}{2}K\langle q^2 \rangle = \frac{1}{2}k_B T$, $K \approx 1.2 \text{ eV/\AA}^2$. The value of the vibronic constant, $f_{a_{2u},a_{1g}} \approx 0.3 \text{ eV}$, was calculated previously (Bersuker et al., 1979). The corresponding "crystal field" matrix element can be calculated using the data on the electronic structure of Fe(P) complexes (Zerner and Gouterman, 1966; Roos and Sundbom, 1970), $v_{a_{2u},a_{1g}} \approx 0.3 \text{ eV}$, and $\delta_{a_{1g},a_{2u}} \approx 0.8 \text{ eV}$ (Zerner and Gouterman, 1966; Eaton et al., 1978). Using all these values, we obtain $q_{ev} \approx 0.16 \text{ \AA}$. Note that both $f_{a_{2u},a_{1g}}$ and $v_{a_{2u},a_{1g}}$ are proportional to the values of the iron and imidazole nitrogen charges. In the calculation we used the charges obtained by the extended Hückel technique, which underestimates charge separation in the complexes (Case et al., 1979). It implies that the calculation underestimates values of q_{ev} .

This estimation unequivocally shows that the electron-vibrational mixing of the $a_{2u}(\pi)$ and $a_{1g}(d_z^2)$ orbitals essentially contributes to the structure of the Fe(P) complexes, steric interaction between the porphyrin and axial ligand also being important. This fact explains qualitatively and semi-quantitatively a number of the experimental data on the iron displacement of the $3d^n$ metalloporphyrin complexes, including its dependence on the kind of the metal, its spin and oxidation state, and the number and nature of the axial ligands (Bersuker and Stavrov, 1988). For example, only electronic reasons can explain the fact that in the complex of the Mn(P) with unhindered 1-methylimidazole (1-MeIm), the metal-porphyrin distance is 0.1 Å longer than in the Fe(P) complex with hindered 2-methylimidazole (2-MeIm). We must notice also that the conclusion (Olafson and Goddard III, 1977) that electronic interactions weakly contribute to the iron out-of-plane displacement, could be a result of a usage of four NH_2 groups in a plane to represent the porphyrin ligand of the Fe(P) molecule. In this model the π conjugated electronic subsystem is absent at all. Consequently, the $a_{2u}(\pi) - a_{1g}(d_z^2)$ mixing which was shown to control the iron out-of-plane displacement in the Fe(P) complexes, in principle could not be taken into account in the study of the $Fe(NH_2)_4$ model.

In this work the influence of the $a_{1g}(d_z^2) - a_{2u}(\pi)$ mixing upon the displacement of the iron, q , on the electronic structure of the compounds under consideration is of particular interest for us. Therefore, first of all, we rewrite Eqs. 2–4, separating the contribution of these two orbitals

$$q_{ev} = \left[F^* + (p_{a_{1g}} - p_{a_{2u}}) f_{a_{2u}, a_{1g}} \frac{v_{a_{2u}, a_{1g}}}{\delta_{a_{1g}, a_{2u}}} \right] \times \left[K^* - (p_{a_{1g}} - p_{a_{2u}}) \frac{f_{a_{2u}, a_{1g}}^2}{\delta_{a_{1g}, a_{2u}}} \right]^{-1} \quad (5)$$

where F^* and K^* include contributions from all the other orbitals. It is possible to see from Eq. 5 that, other things being equal, the greater value of $p_{a_{2u}} - p_{a_{1g}}$ and the smaller that of $\delta_{a_{1g}, a_{2u}}$, the greater is q_{ev} . From Eq. 1 we obtain that the greater is q , the stronger is the $a_{2u}(\pi) - a_{1g}(d_z^2)$ mixture

$$\begin{aligned} \psi_1(q) &= a_{2u}(\pi) - \frac{v_{a_{2u}, a_{1g}}}{2\delta_{a_{1g}, a_{2u}}} a_{1g}(d_z^2) - q \frac{f_{a_{2u}, a_{1g}}}{2\delta_{a_{1g}, a_{2u}}} a_{1g}(d_z^2) \\ \psi_2(q) &= a_{1g}(d_z^2) + \frac{v_{a_{2u}, a_{1g}}}{2\delta_{a_{1g}, a_{2u}}} a_{2u}(\pi) + q \frac{f_{a_{2u}, a_{1g}}}{2\delta_{a_{1g}, a_{2u}}} a_{2u}(\pi) \end{aligned} \quad (6)$$

and the greater are the decreasing of the $\epsilon_1(q)$ and increasing of the $\epsilon_2(q)$, values

$$\begin{aligned} \epsilon_1(q) &= \epsilon_{a_{2u}}(0) - \frac{v_{a_{2u}, a_{1g}}^2}{2\delta_{a_{1g}, a_{2u}}} - q \frac{f_{a_{2u}, a_{1g}} v_{a_{2u}, a_{1g}}}{\delta_{a_{1g}, a_{2u}}} - q^2 \frac{f_{a_{2u}, a_{1g}}^2}{2\delta_{a_{1g}, a_{2u}}} \\ \epsilon_2(q) &= \epsilon_{a_{1g}}(0) + \frac{v_{a_{2u}, a_{1g}}^2}{2\delta_{a_{1g}, a_{2u}}} + q \frac{f_{a_{2u}, a_{1g}} v_{a_{2u}, a_{1g}}}{\delta_{a_{1g}, a_{2u}}} + q^2 \frac{f_{a_{2u}, a_{1g}}^2}{2\delta_{a_{1g}, a_{2u}}} \end{aligned} \quad (7)$$

The direct quantum chemical calculation of the electronic structure of four-coordinated iron porphyrins (Zerner et al.,

1966) showed that the 0.49-Å iron out-of-plane displacement strongly mixes ($\approx 25\%$) the $a_{1g}(d_z^2)$ and $a_{2u}(\pi)$ orbitals, supporting the results of our approach. The origin of the mixing under consideration is illustrated in Fig. 2, wherefrom it is possible to see that the greater is the iron out-of-plane displacement, the greater is the overlap between the iron and the porphyrin MOs.

We used above a rather crude approximation, simulating the effect of the ligand coordination by its "crystal field" only. In reality, the covalent bond between the iron and the axial ligand is also very important. In the case of the imidazole complexes this means that the imidazole binding leads to the substitution of the approximately pure iron or imidazole orbitals by the mixed iron-imidazole MOs. In particular, the interaction between the iron d_z^2 and imidazole nitrogen p_z orbitals leads to the formation of the bonding, mainly imidazole, $\sigma(N_{His} + Fe)$, and antibonding, mainly iron, $\sigma(Fe - N_{His})$, MOs. The energy of the bonding MO is lower than the energy of the corresponding imidazole orbital and, consequently, the energy gap between this MO and the $a_{2u}(\pi)$ one is very large. Since the value of the mixing between any MOs is inversely proportional to the energy gap between them (Eq. 1), the intermixing of the $\sigma(N_{His} + Fe)$ and $a_{2u}(\pi)$ MOs and its contribution to q_{ev} (Eq. 4) is very weak. On the other hand, the strong mixing between the $\sigma(Fe - N_{His})$ and $a_{2u}(\pi)$ orbitals still takes place.

On the basis of this conclusion we can write for the $\psi_1(q)$ and $\psi_2(q)$ MOs

$$\begin{aligned} \psi_1(q) &= (c_{10} - c_{1q}q)a_{2u}(\pi) - (c_{20} + c_{2q}q)\sigma(Fe - N_{His}) \\ \psi_2(q) &= (c_{10} - c_{1q}q)\sigma(Fe - N_{His}) + (c_{20} + c_{2q}q)a_{2u}(\pi), \end{aligned} \quad (8)$$

where the mixing of the orbitals under consideration with other orbitals is neglected, and the c_i coefficients are expanded in powers of the small q displacement, and only the terms up to linear to q is conserved. In this expression the c_{10} describes the static low-symmetry effect of the axial ligand coordination (in the absence of the fifth ligand or in the presence of two equivalent axial ligands $c_{10} = 1$ and $c_{20} = 0$),

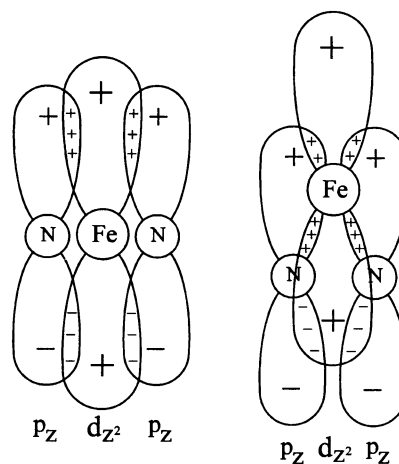


FIGURE 2 Illustration of the origin of the $a_{1g}(d_z^2) - a_{2u}(\pi)$ overlap upon the iron displacement out of the porphyrin nitrogens plane.

and $c_{iq}q$ describes the effect of the iron out-of-plane displacement (at the point $q = 0$ this term vanishes). It is also possible to show from Eq. 5, that c_{i0} and $c_{iq}q$ are of the same sign.

It must be also stressed that in the preceding discussion mainly the electronic causes for the out-of-plane iron displacement in the complexes with one axial ligand were discussed. It is clear that steric repulsion between the axial ligand and the porphyrin ring must increase this displacement, the latter being strongly dependent on the strength of this repulsion. In the particular case of the five-coordinated iron porphyrin complexes with different imidazole ligands, the resulting iron out-of-plane displacement, q_0 , is larger for the sterically hindered imidazoles than for the unhindered ones (Jameson et al., 1980; Momenteau et al., 1988). The R \rightarrow T conformation transition in the Hb also increases the values of q_0 (Perutz et al., 1987; Perutz, 1989). Steric nonbonded interactions can affect the electronic structure of the compound only indirectly through the increase of the iron out-of-plane displacement. To take into account the effect of the steric interactions on the electronic structure we must calculate the electronic structure at the nuclear configuration, corresponding to the real structure of the compound. Since all the dependence of the electronic structure on geometry is described by the Eqs. 1, 2, and 6–8 we can determine the electronic structure of the compound, considering q as a parameter. As it follows from Eqs. 6 and 8, the increase of q due to the steric repulsion must result in a stronger $\sigma(\text{Fe-N}_{\text{His}}) - a_{2u}(\pi)$ mixing.

3. MANIFESTATION OF THE IRON OUT-OF-PLANE DISPLACEMENT IN THE RESONANCE RAMAN SPECTRA OF THE HEME PROTEINS AND THEIR MODELS

According to an earlier paper (Bangcharoenpaupong, 1984) "some of the key Raman observations that must be simultaneously explained by any successful model of the (heme localized) structure function relationship in Hb and Mb: 1) strong coupling of the Fe-N_{His} mode to the Soret resonance of deoxy Mb and deoxy Hb; 2) higher Fe-N_{His} mode frequency in the deoxy R state than in the deoxy T state; 3) inverse relationship between changes in the Fe-N_{His} mode frequency and porphyrin ring breathing mode, ν_4 ; 4) weaker Fe-N_{His} Raman scattering (approximately a factor of two) from the deoxy T state than from the deoxy R state Hb or deoxy Mb; 5) weak (or vanishing) coupling of the Fe-N_{His} mode in oxy Hb, Mb (no Fe-N_{His} mode has been definitively assigned in *any* ligated complex); and 6) no change in the Fe-O₂ stretching frequency (or other Fe-ligand frequencies) between R and T forms of Hb." Up till now all these items, except the fourth one, are the experimental criteria to be satisfied by any successful theoretical model of the structure-function relationship in Hb and Mb (see, for example, Rousseau and Friedman (1988)). As far as the fourth criterion is concerned, the latest investigations (Nagai and Kitagawa, 1980; Nagai et al., 1980; Sassaroli et al., 1986; Kitagawa,

1988; Friedman et al., 1990; Ahmed et al., 1991; Bosenbeck et al., 1992) showed that there is a more complicated relationship between the protein conformation and the intensity of the Fe-N_{His} Raman band. In this section we will show that the systematic study of the effect of the iron out-of-plane displacement in the heme proteins and their models can successfully explain all the experimental data.

Frequencies

We shall first consider the relationships between the different ground state properties of the compounds under consideration, such as their structure and vibrational frequencies. To understand the influence of the mixing discussed above on $\nu(\text{Fe-N}_{\text{His}})$ we must recall that the $\sigma(\text{Fe-N}_{\text{His}})$ MO is the antibonding iron-histidine orbital. Consequently, the greater is its population, the smaller must be the Fe-N_{His} frequency and the greater – the Fe-N_{His} distance. From Eq. 8 we can obtain an expression for the population of the $\sigma(\text{Fe-N}_{\text{His}})$ MO, p_σ

$$p_\sigma(q_0) = p_1 + (p_1 - p_2)(2c_{i0}c_{iq}q_0 - c_{i0}^2) \quad (9)$$

where p_1 and p_2 are the population numbers of the ψ_1 and ψ_2 MOs, respectively. In the high- and intermediate-spin ground states $p_1 = 2$, and $p_2 = 1$, and in the low-spin ground state $p_2 = 0$. Therefore, in the ground state $p_1 - p_2 \geq 0$, and an increase of the iron out-of-plane displacement increases the population of the $\sigma(\text{Fe-N}_{\text{His}})$ MO. Hence, the Fe-N_{His} frequency must decrease upon increase of the iron out-of-plane displacement. This relationship between q_0 and $\nu(\text{Fe-N}_{\text{His}})$ was already observed experimentally (Desbois and Lutz, 1981; Desbois et al., 1981; Argade et al., 1984). This result is also in excellent agreement with the results of the resonance Raman and x-ray diffraction studies of the model compounds (Table 1), wherefrom one can see, that an increase of the iron out-of-plane displacement systematically decreases the $\nu(\text{Fe-N}_{\text{His}})$ value.

At the same time it is very difficult, if at all possible, to explain the changes of the Fe-N_{His} frequencies taking into consideration only the $e_g(\pi^*) - \sigma(\text{Fe-N}_{\text{His}})$ mixing (Bangcharoenpaupong, 1984). Indeed, an increase of the tilt angle value, θ , increases the overlap between the empty $e_g(\pi^*)$ and the partially populated antibonding $\sigma(\text{Fe-N}_{\text{His}})$ orbitals, decreasing the population of the latter, which, as it follows from the BSC model, must increase the $\nu(\text{Fe-N}_{\text{His}})$ value. Comparing the data of the Table 1 for the iron porphyrin complexes with 2-MeIm or 1-MeIm, we see that very pronounced opposite changes take place. In general, substitution of 2-MeIm for 1-MeIm increases the θ angle (Jameson et al. 1980; Bangcharoenpaupong, 1984; Momenteau et al., 1988) and essentially reduces the Fe-N_{His} frequency, for example, it equals to 227 and 215 cm^{-1} in FePocPiv(1-MeIm) and FePocPiv(2-MeIm), respectively (Hori and Kitagawa, 1980; Collman et al., 1983). These data imply that in the compounds under consideration the mixing of $\sigma(\text{Fe-N}_{\text{His}})$ and $e_g(\pi^*)$ orbitals is weaker than the mixing of $\sigma(\text{Fe-N}_{\text{His}})$ and $a_{2u}(\pi)$ MOs and, as a result, the features of the Fe-N_{His}

Table 1 The structures and Fe-N_{His} and ν_4 frequencies of some imidazole complexes of ferroporphyrins.

	θ	ϕ	Fe-N _{Im}	Fe-P _N	$\nu(\text{Fe-N}_{\text{His}})$	ν_4
	degrees		\AA		cm^{-1}	
Fe(Piv ₂ C ₈)(1-MeIm)	5.0*	9.2*	2.13 (2)*	0.31 (2)*	225 [†] – 227 [§]	1355 [†]
Fe(TPivPP)(2-MeIm)	9.5	22.8	2.09	0.40	209 [¶]	1345 ^{**}
Fe(TPP)(2-MeIm)	10.2	7.4	2.16	0.42	206 ^{**}	

* Momenteau et al., 1988.

[†] Fe(TPivPP)(1-MeIm) (Hori and Kitagawa, 1980).[§] Fe(PocPiv)(1-MeIm) (Collman et al., 1983).^{||} Jameson et al. (1980).[¶] Hori and Kitagawa (1980).^{**} Fe(TPivPP)(1,2-Me₂Im) (Hori and Kitagawa, 1980).^{**} Collman et al. (1983).

bond depend mainly on the latter mixing. This conclusion was supported by our very recent quantum chemical calculations (Stavrov and Kushkuley, 1993), which show that the strength of the Fe-N_{His} bond is controlled mainly by the iron out-of-plane displacement, the dependence on the azimuthal angle value, ϕ , being negligibly weak at different values of θ between 0° and 20°. The tilt also affects the Fe-N_{His} bond but rise of θ by 10° increases the strength of this bond approximately two times weaker than rise of q_0 by 0.1 Å decreases it.

As far as the Fe-N_{His} distance is considered, we can conclude from Table 1, that this distance does not depend monotonically on the q_0 . One of the possible reasons for this complicated dependence is that the steric repulsion affects the Fe-N_{His} bond both directly, elongating the Fe-N_{His} distance, and indirectly, changing the iron out-of-plane displacement, which also increases the distance under consideration.

The present approach also explains the higher Fe-N_{His} frequency in the deoxy R state than in the deoxy T state of Hb (criterion 2) as a result of the increase of the iron out-of-plane displacement in the latter due to the protein globule tension (Perutz et al., 1987; Perutz, 1989). Note, however, that one must be very careful in comparing the $\nu(\text{Fe-N}_{\text{His}})$ values for different types of heme proteins, because other mechanisms, such as weak hydrogen bond formation, can affect this frequency very pronouncedly (Teraoka and Kitagawa, 1981; Kitagawa, 1988). For example, on the proximal side of the heme pocket of sperm whale Mb there is a serine side chain, that is absent in Hb (Lesk and Chotia, 1980), and that can form an additional hydrogen bond to the proximal histidine (Takano, 1977a, 1977b). This bond can be responsible, at least partially, for the different temperature dependence (Rousseau and Friedman, 1988) of the (Fe-N_{His}) band in Mb than in Hb.

The fact, that the increase of q_0 reduces the Fe-N_{His} frequency, explains also the decrease of this frequency with time after the flash-photolysis of diatomic complexes of Hb (Rousseau and Friedman, 1988) as a manifestation of the incomplete relaxation of the heme (smaller q_0 values, than in deoxy Hb) immediately after photolysis and the relatively slow movement of the iron atom to its stable out-of-plane position. This explanation is also consistent with the analysis of the time (Murray et al., 1988) and temperature (Nienhaus

et al., 1992) evolution of the near infrared 760-nm absorption band and of the frequencies of the porphyrin vibrations of Hb after photolysis (Dasgupta and Spiro, 1986; Rousseau and Friedman, 1988).

The value of the ν_4 frequency is controlled by the population of the $e_g(\pi^*)$ MO (Spiro, 1988). This orbital is an antibonding orbital with respect to the pyrrole C—N bond of the porphyrin ring (Gouterman, 1959, 1978; Bangcharoenpaupong, 1984; Spiro, 1988). Its depopulation increases the C—N force constant, increasing the frequency of the porphyrin ring breathing ν_4 vibration (Spiro, 1988). The displacement of the iron out of the porphyrin plane reduces the overlap between the iron $e_g(d_\pi)$ and the porphyrin $e_g(\pi^*)$ MOs, reducing the charge transfer to the porphyrin $e_g(\pi^*)$ orbital and, consequently, increasing the ν_4 frequency. Therefore, any change of the iron out-of-plane displacement per se must lead to the inverse relationship between changes of the frequencies of this mode and of the Fe-N_{His} one (criterion 3). However, the maximal absolute value of the admixing of the $e_g(d_\pi)$ MO to the $e_g(\pi^*)$ one is extremely small, ≈ 0.02 (Zerner and Gouterman, 1966), and, consequently, its reduction upon the iron out-of-plane displacement cannot be strong. Therefore, the imidazole tilt, considered in the BSC model, can affect the ν_4 frequency more pronouncedly. Because of small $e_g(\pi^*) - e_g(d_\pi)$ mixing even weak charge transfer from $\sigma(\text{Fe-N}_{\text{His}})$ to $e_g(\pi^*)$ must increase significantly the population of the $e_g(\pi^*)$ MO, reducing the ν_4 value relatively strongly. It is the increase of the $\sigma(\text{Fe-N}_{\text{His}}) \rightarrow e_g(\pi^*)$ charge transfer on going from Fe(TPivPP)(1-MeIm), $\theta \approx 5^\circ$, to Fe(TpivPP)(1-, 2-Me₂Im), $\theta \approx 10^\circ$, that may explain the reduction of the ν_4 frequency from 1355 to 1345 cm^{-1} in these compounds (Hori and Kitagawa, 1980). In the model compounds under consideration the increase of the steric repulsion due to the substitution of 2-MeIm for 1-MeIm increases both the iron out-of-plane displacement and the imidazole tilt (Jameson et al., 1980; Momenteau et al., 1988), decreasing both the $\nu(\text{Fe-N}_{\text{His}})$ and ν_4 frequencies. The protein can affect the histidine coordination geometry in another way, by changing mainly q_0 , and only slightly the tilt angle, θ . This must lead to the inverse relationship between the changes of the $\nu(\text{Fe-N}_{\text{His}})$ and ν_4 frequencies, the changing of the ν_4 frequency being much smaller, than

in the methylimidazole containing model compounds. The dependence of these frequencies on several parameters can explain also their different relaxation kinetics in the photo-lyzed carbon monoxide complex of Hb (Rousseau and Friedman, 1988; Dasgupta and Spiro, 1986). Note also, that the $\sigma(\text{Fe-N}_{\text{His}}) \rightarrow e_g(\pi^*)$ charge transfer can take place only in the compounds with populated $\sigma(\text{Fe-N}_{\text{His}})$ MO. Therefore, we can expect lower ν_4 values in the high-spin iron compounds ($\sigma(\text{Fe-N}_{\text{His}})$ orbital is populated), than in the low-spin ones ($\sigma(\text{Fe-N}_{\text{His}})$ MO is empty), this conclusion being in agreement with the experimental results (Spiro, 1983).

All the results on the dependence of the Fe-N_{His} frequency on the heme structure prove that the iron displacement out of the porphyrin plane is the parameter which mainly controls this frequency (provided, obviously, that the protonation of the imidazole is not changed). Taking advantage from this result, we can use the data from the Table 1 to find an approximate (without taking into account of the effect of the simultaneous tilt of the imidazole) correlation between the q_0 and $\nu(\text{Fe-N}_{\text{His}})$ values:

$$\nu (\text{cm}^{-1}) = 209.4 - 184.0[q_0(\text{\AA}) - 0.4] \quad (10)$$

Coordination of the second axial ligand dramatically increases the energy gap between the $\sigma(\text{Fe-N}_{\text{His}})$ and $a_{2u}(\pi)$ orbitals (Eaton et al., 1978; Gouterman, 1978; Bersuker and Stavrov, 1988) and essentially reduces the low-symmetry axial "crystal field," causing the iron to move closer to the porphyrin plane (Bersuker and Stavrov, 1988). All these effects strongly reduce the mixing between the $a_{2u}(\pi)$ and σ orbitals, the latter now carrying contributions of the d_{z^2} iron orbital and the σ orbitals of both axial ligands. Therefore in the hexacoordinated compounds the nature of the σ orbital is controlled mainly by the iron-axial ligand distance, which in proteins weakly depends on their conformation (Liddington et al., 1988), and in model compounds on the steric interaction with the fifth ligand (Jameson et al., 1980; Momenteau et al., 1988). That is why the trans-effect of the fifth ligand coordination geometry depends only weakly on the protein conformation, which can explain the weakness or absence of change of the Fe-O_2 vibrational frequency upon the changing the protein conformation (criterion 6).

Intensities

The first, fourth, and fifth criteria mentioned above deal with the intensity of the Fe-N_{His} band upon excitation in the Soret band region. The considerable intensity of the Fe-N_{His} band implies that the characteristics of the adiabatic surfaces (the iron-imidazole distance, force field constant, etc.) of the ground state, GS, and the corresponding excited B state, must be different. This means, in turn, that in the B state the electronic population of the $\sigma(\text{Fe-N}_{\text{His}})$ orbital is notably changed. The necessary condition for this, is a participation of the $\sigma(\text{Fe-N}_{\text{His}})$ orbital in the excitation. Such a participation really takes place in the compounds with one axial

ligand due to the mixing of the $a_{2u}(\pi)$ and $\sigma(\text{Fe-N}_{\text{His}})$ orbitals. Coordination of the second axial ligand, as it was mentioned, strongly reduces the mixing between these orbitals, leading to the weak or vanishing coupling of the Fe-N_{His} mode to the Soret resonance (criterion 5).

To prove, that in the five-coordinated compounds the intensity of the Fe-N_{His} band is strong enough to be observable, we must show, that the electronic populations of the $\sigma(\text{Fe-N}_{\text{His}})$ orbital in the ground and the B states are essentially different. According to the four-orbital model (Gouterman, 1959, 1978) any component of the doubly degenerated B state of the iron-porphyrin complex can be written as the sum of two determinants

$$B_{x(y)} = \frac{1}{\sqrt{2}} [|\psi_1^{(1)}e_{gx(y)}^{(1)}| + |a_{1u}^{(1)}e_{gy(x)}^{(1)}|] \quad (11)$$

where only the MOs whose populations change upon the excitation are listed and the mixing between the $a_{2u}(\pi)$ and $\sigma(\text{Fe-N}_{\text{His}})$ orbitals is already taken into account. It is simple to see from Eq. 11 that in the B state the ψ_1 electronic population, p_1 , equals to 1.5 instead of 2 in the ground state, the p_2 value remaining the same, $p_2 = 1$. Keeping this in mind and using Eq. 9 we can write for the $\Delta p_\sigma = p_\sigma(\text{B}) - p_\sigma(\text{GS})$:

$$\Delta p_\sigma = -0.5[2c_{10}c_{1q}(2q_0^{\text{GS}} - q_0^{\text{B}}) - c_{10}^2] \quad (12)$$

where $q_{1u}^{(1)}$ and $q_{1u}^{(1)}$ are the iron out-of-plane displacement in the ground and B states. Assuming that these displacements are equal $q_0^{\text{GS}} = q_0^{\text{B}} = q_0$ we can rewrite Eq. 12

$$\Delta p_\sigma = -0.5(2c_{10}c_{1q}q_0 - c_{10}^2). \quad (13)$$

Using results (Zerner et al., 1966) it is possible to estimate the population values for $q_0 = 0.49 \text{ \AA}$: $p_\sigma(\text{GS}) \approx 1.25$, $p_\sigma(\text{B}) \approx 1.12$, and $\Delta p_\sigma \approx -0.13$. We see that the population of the $\sigma(\text{Fe-N}_{\text{His}})$ MO is reduced essentially by the Soret excitation, which causes the reduction of the Fe-N_{His} distance and force field constant, leading to the strong coupling of the Fe-N_{His} vibration to the Soret resonance (criterion 1).

It also follows from Eq. 13, that the greater is q_0 in the ground state, the greater is Δp_σ and, consequently, the greater must be the Fe-N_{His} band intensity. It means that the smaller is the frequency of the Fe-N_{His} band, the greater must be its intensity. Experimental data, however, show no monotonic dependence between the frequency and intensity of the Fe-N_{His} band (Nagai and Kitagawa, 1980; Nagai et al., 1980; Sassaroli et al., 1986; Rousseau and Friedman, 1988; Friedman et al., 1990; Ahmed et al., 1991; Bosenbeck et al. 1992). In general this experimental fact means, that the frequency and intensity of the Fe-N_{His} band are governed not only by the ground state q_0 value but by other factors, too. It was mentioned above, that the hydrogen bond between the proximal histidine and the protein globule can affect the Fe-N_{His} frequency: the stronger the bond, the higher is the frequency. Therefore, if upon the out-of-plane iron movement a strengthening of the hydrogen bond takes place, it can compensate the reduction of the Fe-N_{His} frequency and only

weakly affect the corresponding intensity, leading to the apparent absence of relationship between the frequency and intensity. A tilt of the imidazole can be considered as another parameter, which increases both the frequency and the intensity. From this point of view a lack of dependence between the position and the intensity of the Fe-N_{His} band in different heme proteins (Friedman et al., 1990) can be caused by a simultaneous change of the iron out-of-plane displacement and the histidine tilt. In this case we still must assume, that the histidine tilt is changed significantly more, or the iron displacement is changed significantly less, than in the model compounds, where a reduction of the Fe-N_{His} frequency due to the increase of q_0 strongly exceeds its decrease due to the tilt (see Table 1). This hypothesis, however, does not agree with the inverse relationship between the Fe-N_{His} and ν_4 frequencies, discussed above. Friedman et al., 1990 proposed an azimuthal angle ϕ (Fig. 1) controlling the overlap between the iron $4p_\pi$ and imidazole π_{Im} orbitals as a parameter, governing the intensity and not the frequency. This assumption does not look well-founded, because the change of the $4p_\pi$ - π_{Im} overlap population upon the ϕ change from 0° to 45° is very small itself being less than $0.01 e^-$ (Scheidt and Chipman, 1986). The Fe-N_{His} band intensity is affected by the mixing of the porphyrin and iron-imidazole electron subsystems and, consequently, the intensity is governed by the change of overlap population between the imidazole π_{Im} and porphyrin π orbitals. This population depends on ϕ even weaker than the $4p_\pi$ - π_{Im} overlap population, because it is proportional to the latter one multiplied by the very weak overlap between the iron $4p_\pi$ and the porphyrin $e_g(\pi^*)$ orbitals.

All the parameters discussed above are the ground state parameters of the compounds under consideration. At the same time there is one more possible reason for the complicated relationship between the Fe-N_{His} frequency and intensity. We must recall, that the expression (Eq. 13) was obtained under the assumption that q_0 is the same in the ground and B states. To test this assumption we must consider the expression (Eq. 5) for the B state. Using Eq. 11 for the iron-in-plane nuclear configuration we can easily show, that in this state the effective population of the $a_{2u}(\pi)$ MO is 1.5, instead of 2 in the ground state. Since q_{ev} decreases upon a decrease in the $p_{a_{2u}} - p_{a_{1g}}$ value (Eq. 5), it follows that due to the electronic causes the $q_{ev}^{GS} > q_{ev}^{GS}$. If the q_0 had been controlled only by electronic effects this difference could only increase Δp_{σ} , increasing the Fe-N_{His} band intensity. However, it was noted in Section 2 that the steric repulsion or/and protein tension can considerably increase the q_0 value comparing to the q_{ev} one. These interactions reduce the approaching of the iron atom to the porphyrin plane in the excited state and, consequently, reduce the Fe-N_{His} band intensity. Thus, in compounds where the steric interactions play an important role, the intensity under consideration can be smaller than in compounds where the steric interactions are weak enough.

This conclusion can be proved using Eq. 12. Let us consider first the compounds where the steric interactions be-

tween the axial ligand and the porphyrin are weak enough to be neglected, r compounds. For the sake of simplicity we will take into account only a contribution of mixing the $a_{1g}(d_z^2)$ and $a_{2u}(\pi)$ orbitals into the q_{ev} value, $F^* = 0$ (5). In this case the iron out-of-plane displacement is controlled only by the electronic interactions, $q_{0,r} = q_{ev}$, and is proportional to $p_{a_{2u}} - p_{a_{1g}}$. Consequently, $q_{0,r}^B = \frac{1}{2}q_{0,r}^{GS}$, and we obtain from Eq. 12

$$\Delta p_{\sigma,r} = -0.5(3c_{10}c_{1q}q_{0,r}^{GS} - c_{10}^2). \quad (14)$$

In the opposite situation (t compounds) when the steric interactions are much stronger than the electronic ones, the change of the iron out-of-plane displacement upon the excitation of the system into the B state is negligibly small. In this case the value of $\Delta p_{\sigma,t}$ is determined by Eq. 13. An expression for the difference between the absolute values of the $\Delta p_{\sigma,r}$ and $\Delta p_{\sigma,t}$ can be obtained from Eqs. 13 and 14,

$$|\Delta p_{\sigma,r}| - |\Delta p_{\sigma,t}| = c_{10}c_{1q}(\frac{3}{2}q_{0,r}^{GS} - q_{0,t}^{GS}), \quad (15)$$

and can be positive even if $q_{0,t}^{GS} > q_{0,r}^{GS}$. Consequently, the intensity of the Fe-N_{His} band can be greater in the r compound than in the t one even if $q_{0,t}^{GS} > q_{0,r}^{GS}$. If in both compounds the steric interactions are strong (both compounds are of t type (Eq. 13)) or weak (both compounds are of r type (Eq. 14)) the larger q_0^{GS} is the stronger must be the Fe-N_{His} intensity. Certainly, upon the elaboration of the expressions (Eqs. 12–15) a quite crude approximation was used. In real complexes the intermediate situation takes place and the relationship between the q_0^{GS} and the intensity and, consequently, between the Fe-N_{His} frequency and intensity, can be very complicated.

The contribution of the steric and electronic interactions to the value in the different protein conformations can be estimated from the comparison of the ground state Fe-N_{His} vibrational frequencies. The small reduction of $\nu(\text{Fe-N}_{His})$ upon the R \rightarrow T conformational transition implies that the protein tension is weak in both conformations. Consequently, the electronic effects prevail in both T and R conformations (both compounds are of the r type) and the Fe-N_{His} band intensity must be greater in the T conformation, than in the R one. Significant reduction of $\nu(\text{Fe-N}_{His})$ upon the R \rightarrow T conformational transition means that the opposite situation occurs, the protein tension in the T conformation is strong and essentially contributes to the iron out-of-plane displacement (t type compound), in the R conformation the q_0 still being mainly controlled by the electronic effects (r type compound). In this case the Fe-N_{His} intensity can be stronger in the R conformation than in the T one. For example, the R conformation of the valency hybrid Hb $\alpha_2^{+CN}\beta_2^{\text{deoxy}}$ can be changed to the T one by decreasing the pH value from 9.0 to 6.5 and addition of inositol hexaphosphate (Nagai and Kitagawa, 1980). This conformational transition slightly reduces the $\nu(\text{Fe-N}_{His})$ value from 224 to 220 cm^{-1} , implying that the protein tension in the T conformation is weak, the electronic effects prevail (both conformations belong to the r type compounds) and the Fe-N_{His} band intensity must be

stronger in the T conformation of the protein: this is really the case (Nagai and Kitagawa, 1980). The opposite situation takes place in another valency hybrid Hb, $\alpha_2^{\text{deoxy}}\beta_2^{\text{+CN}}$, which shows a strong shift of the $\nu(\text{Fe-N}_{\text{His}})$ from 222 to 207 cm^{-1} on going from the R to T conformation. This means that in the T conformation the protein tension is rather strong (t compound), the R conformation being of the r type. This conclusion explains the higher intensity of the Fe-N_{His} band in the R conformation of the protein than in the T one (Nagai and Kitagawa, 1980).

In general the complicated dependence of the Fe-N_{His} band intensities on the protein conformation can be explained as the result of the interplay between the electronic and steric (or protein tension) effects in the systems under consideration. From this point of view the extent and direction of the imidazole tilt can be very important, because they can change dramatically the steric interaction between the histidine and the porphyrin plane, essentially affecting the Fe-N_{His} band intensity.

It follows also from Eq. 7 that an increase of q lowers ϵ_1 energy of the ψ_1 orbital. At the same time the Q and Soret absorption bands of metalloporphyrins correspond to the ψ_1 , $a_{1u}(\pi) \rightarrow e_g(\pi^*)$ excitations strongly mixed by the configuration interaction (Gouterman, 1959, 1978). Therefore, if the $e_g(\pi^*)$ energy weakly depends on q , the decrease of the ϵ_1 must lead to the blue shift of both bands. The imidazole tilt increases the energy of the $e_g(\pi^*)$ MO also leading to the blue shift of the B and Q bands (Bangcharoenpaupong et al., 1984). Such a blue shift was indeed observed in the Fe-(PocPiv)(Im) complexes upon the substitution of 2-MeIm for 1-MeIm (Collman et al., 1983), and on the R \rightarrow T conformational transition of Hb (Perutz et al., 1974).

4. CONCLUSION

In this paper the influence of the iron displacement out of the plane of the porphyrin nitrogens in heme proteins and iron porphyrin complexes on their electronic structure was studied using the vibronic theory. It was shown that this displacement crucially mixes the iron-imidazole and porphyrin electronic subsystems, leading to the strong coupling of the Fe-N_{His} vibration to the porphyrin $\pi \rightarrow \pi^*$ excitations. The spectroscopic and structural manifestations of the theoretical results, obtained in this work, are in a good agreement with a number of experimental data, which stresses the crucial role of the iron out-of-plane displacement for the understanding of the features of the systems under consideration.

It also follows from our analysis that the tilt of the imidazole ligand itself can not explain a very important piece of the experimental data: the relationship between the Fe-N_{His} frequency and the geometry of the model compounds. Therefore we can state that, as far as the heme geometry is considered, the features of the resonance Raman Fe-N_{His} band of the compounds of interest depend mainly on the value of the iron out-of-plane displacement, the imidazole tilt being able to modulate this dependence mostly indirectly,

changing the iron displacement. Thus, the position of the resonance Raman Fe-N_{His} band of heme proteins in different conformations contains mainly information on the dependence of the iron out-of-plane displacement on the conformation of the protein, which was shown to be very important for the understanding of the binding of small molecules to heme proteins (Perutz et al., 1987; Liddington et al., 1988; Šrajter et al., 1988).

All the obtained results are qualitative or semiquantitative. The direct quantum chemical calculations of the dependence of the electronic structure and vibrational frequencies on the iron out-of-plane displacement and geometry of the imidazole coordination in the compounds under consideration, are now in progress.

This work was supported by the Sackler Fund for Scientists Absorption and the Center for Scientists Absorption, Ministry of Absorption, State of Israel.

REFERENCES

- Ahmed, A. M., B. F. Campbell, D. Caruso, M. R. Chance, M. D. Chavez, S. H. Courtney, J. M. Friedman, I. E. T. Iben, M. R. Ondrias, and M. Yang. 1991. Evidence for proximal control of ligand specificity in heme proteins: absorption and Raman studies of cryogenically trapped photoproducts of ligand bound myoglobins. *Chem. Phys.* 158: 329–351.
- Argade, P. V., M. Sassaroli, D. L. Rousseau, T. Inubushi, M. Ikeda-Saito, and A. Lapidot. 1984. Confirmation of the assignment of the iron-histidine stretching mode in myoglobin. *J. Am. Chem. Soc.* 106:6593–6596.
- Bangcharoenpaupong, O., K. T. Schomaker, and P. M. Champion. 1984. A resonance Raman investigation of myoglobin and hemoglobin. *J. Am. Chem. Soc.* 106:5688–5698.
- Bosenbeck, M., R. Schweitzer-Stenner, and W. Dreybrodt. 1992. pH-induced conformational changes of the Fe²⁺-N_ε(His F8) linkage in deoxyhemoglobin trout IV detected by the Raman active Fe²⁺-N_ε(His F8) stretching mode. *Biophys. J.* 61:31–41.
- Bersuker, I. B., S. S. Stavrov, and B. G. Vekhter. 1979. Pseudo Jahn-Teller effect as a cause of the iron atom displacement from the heme group plane and possible source of trigger mechanism of conformational changes during oxygenation of hemoglobin. *Biophysika (Biophysics, Moscow)*. 24:413–418.
- Bersuker, I. B., and S. S. Stavrov 1981. Vibronic effects in geometry and stereochemistry of metalloporphyrins and hemoproteins. *Chem. Physics*. 54:331–340.
- Bersuker, I. B., and S. S. Stavrov 1983. Spin state/stereochemistry relationships in metalloporphyrins and hemoproteins: the vibronic approach. *Inorg. Chim. Acta.* 79:177.
- Bersuker, I. B., and S. S. Stavrov 1988. Structure and properties of metalloporphyrins and hemoproteins: the vibronic approach. *Coord. Chem. Rev.* 88:1–68.
- Case, D. A., B. H. Huynh, and M. Karplus. 1979. Binding of oxygen and carbon monoxide to hemoglobin. An analysis of the ground and excited states. *J. Am. Chem. Soc.* 101:4433–4453.
- Champion, P. M. 1988. Cytochrome P450 and the transform analysis of heme protein Raman spectra. In *Biological Application of Raman Spectroscopy*. T. G. Spiro, editor. Wiley & Sons, New York. Vol. III, 249–292.
- Collman, J. P., J. I. Brauman, T. J. Collins, B. L. Iverson, G. Lang, R. B. Pettman, J. L. Sessler, and M. A. Walters. 1983. Synthesis and characterization of the "pocket" porphyrins. *J. Am. Chem. Soc.* 105:3038–3052.
- Dasgupta, S., and T. G. Spiro. 1986. Resonance Raman characterization of the 7-ns photoproduct of (carbonmonoxy)hemoglobin: implications for hemoglobin dynamics. *Biochemistry*. 25:5941–8.
- Desbois, A., and M. Lutz. 1981. Low-frequency vibrations of ferroporphyrin-substituted imidazole complexes. *Biochim. Biophys. Acta.* 671:168–176.

- Desbois, A., M. Lutz, and R. Banerjee. 1981. Protoheme conformations in low-spin ferrohemoproteins. Resonance Raman spectroscopy. *Biochim. Biophys. Acta.* 671:184-192.
- Eaton, W. A., L. K. Hanson, P. J. Stephens, J. C. Sutherland, and J. B. R. Dunn. 1978. Optical spectra of oxy- and deoxyhemoglobin. *J. Am. Chem. Soc.* 100:4991-5003.
- Frauenfelder, H., F. Parak, and R. D. Young. 1988. Conformational sub-states in proteins. *Annu. Rev. Biophys. Biochem.* 17:451-79.
- Friedman, J. M., D. L. Rousseau, and M. R. Ondrias. 1982. Time-resolved resonance Raman studies of hemoglobin. *Annu. Rev. Phys. Chem.* 33: 471-491.
- Friedman, J. M., B. F. Campbell, and R. W. Noble. 1990. A possible new control mechanism suggested by resonance Raman spectra from a deep ocean fish hemoglobin. *Biophys. Chem.* 37:43-59.
- Gelin, B. R., A. W.-M. Lee, and M. Karplus. 1983. Hemoglobin tertiary structural change on ligand binding. Its role in the co-operative mechanism. *J. Mol. Biol.* 171:489-559.
- Gouterman, M. 1959. Study of the effects of substitution on the absorption spectra of porphyrin. *J. Chem. Phys.* 30:1139-1161.
- Gouterman, M. 1978. Optical spectra and electronic structure of porphyrins and related rings. In *The Porphyrins*. D. Dolphin, editor. Academic, New York. Vol. III, 1-165.
- Hori, H., and T. Kitagawa. 1980. Iron-ligand stretching band in the resonance Raman spectra of ferrous iron porphyrin derivatives. Importance as a probe band for quaternary structure of hemoglobin. *J. Am. Chem. Soc.* 102:3608-3613.
- Jameson, G. B., F. S. Molinaro, J. A. Ibers, J. P. Collman, J. I. Brauman, E. Rose, and K. S. Suslick. 1980. Models for the active site of oxygen-binding hemoproteins. dioxygen binding properties and the structures of (2-methylimidazole)-*meso*-tetra($\alpha, \alpha, \alpha, \alpha$ -*o*-pivalamidophenyl)porphyrinatoiron(II)-ethanol and its dioxygen adduct. *J. Am. Chem. Soc.* 102: 3224-3237.
- Kitagawa, T. 1988. The heme protein structure and the iron histidine stretching mode. In *Biological Application of Raman Spectroscopy*. T. G. Spiro, editor. Wiley & Sons, New York. Vol. III, 97-131.
- Lesk, A. M., and C. Chothia. 1980. How different amino acid sequences determine similar protein structures: the structure and evolutionary dynamics of the globins. *J. Mol. Biol.* 136:225-70.
- Li, X.-Y., and Z. Zgierski. 1992. Iron motion in a five-coordinated heme model. *Chem. Phys. Lett.* 188:16-20.
- Liddington, R., Z. Derewenda, G. Dodson, and D. Harris. 1988. Structure of the liganded T state of haemoglobin identifies the origin of cooperative oxygen binding. *Nature (Lond.)* 331:725-728.
- Momenteau, M., W. R. Scheidt, C. W. Eigenbrot, and C. A. Reed. 1988. A deoxymyoglobin model with a sterically unhindered axial imidazole. *J. Am. Chem. Soc.* 110:1207-1215.
- Murray, L. P., J. Hofrichter, E. R. Henry, and W. A. Eaton. 1988. Time-resolved optical spectroscopy and structural dynamics following photodissociation of carbonmonoxyhemoglobin. *Biophys. Chem.* 29: 63-76.
- Nagai, K., and T. Kitagawa. 1980. Differences in Fe(II)-N₆(His-F8) stretching frequencies between deoxyhemoglobins in the two alternative quaternary structures. *Proc. Natl. Acad. Sci. U.S.A.* 77:2033-2037.
- Nagai, K., T. Kitagawa, and H. Morimoto. 1980. Quaternary structures and low frequency molecular vibrations of haems of deoxy and oxyhaemoglobin studied by resonance Raman scattering. *J. Mol. Biol.* 136: 271-289.
- Nienhaus, G. U., J. R. Mourant, and H. Frauenfelder. 1992. Spectroscopic evidence for conformational relaxation in myoglobin. *Proc. Natl. Acad. Sci. U.S.A.* 89:2902-2906.
- Olafson, B. D., and Goddard III, W. A. 1977. Molecular description of dioxygen bonding in hemoglobin. *Proc. Natl. Acad. Sci. U.S.A.* 74: 1315-1319.
- Parak, F., E. W. Knapp, and D. Kucheida. 1982. Protein dynamics. Mössbauer spectroscopy on deoxymyoglobin crystals. *J. Mol. Biol.* 161: 177-194.
- Perutz, M. F., J. E. Ladner, S. R. Simon, and C. Ho. 1974. Influence of globin structure on the state of the heme. I. Human deoxyhemoglobin. *Biochemistry.* 13:2163-2173.
- Perutz, M. F., G. Fermi, B. Luisi, B. Shaanan, and R. C. Liddington. 1987. Stereochemistry of cooperative mechanisms in hemoglobin. *Acc. Chem. Res.* 20:309-321.
- Perutz, M. F. 1989. Mechanisms of cooperativity and allosteric regulation in proteins. *Quart. Rev. Biophys.* 22:139-236.
- Roos, B., and M. Sundbom. 1970. A theoretical investigation of the electronic structure and excited states of copper porphyrin. *J. Mol. Struct.* 36: 8-25.
- Rousseau, D. L., and J. M. Friedman. 1988. Transient and cryogenic studies of photodissociated hemoglobin and myoglobin. In *Biological Application of Raman Spectroscopy*. T. G. Spiro, editor. Wiley & Sons, New York. Vol. III, 133-215.
- Sassaroli, M., S. Dasgupta, and D. L. Rousseau. 1986. Cryogenic stabilization of myoglobin photoproducts. *J. Biol. Chem.* 261:13704-13713.
- Scheidt, W. R., and D. M. Chipman. 1986. Preferred orientation of imidazole ligands in metalloporphyrins. *J. Am. Chem. Soc.* 108:1163-1167.
- Spiro, T. G. 1983. The resonance Raman spectroscopy of metalloporphyrins and heme proteins. In *Iron Porphyrins*. Part II. A. B. P. Lever and H. B. Gray, editors. Addison-Wesley, Reading, PA. 89-158.
- Spiro, T. G. 1988. Resonance Raman spectroscopy of metalloporphyrins. In *Biological Application of Raman Spectroscopy*. T. G. Spiro, editor. Wiley & Sons, New York. Vol. III, 1-37.
- rager, V., L. Reinisch, and P. M. Champion. 1988. Protein fluctuations, distributed coupling, and the binding of ligands to heme proteins. *J. Am. Chem. Soc.* 110:6656-6670.
- Stavrov, S. S., and I. B. Bersuker. 1983. Ligand coordination effects in spin state/stereochemistry relationships in metalloporphyrins and hemoproteins. *Inorg. Chim. Acta.* 79:176.
- Stavrov, S. S. 1992. The contribution of the out-of-plane iron displacement to the strong coupling of the Fe-N(His F8)-mode in the resonance region of the Soret band. In *Abstracts of the Symposium on Raman Spectroscopy on Biological Systems*. University of Bremen, September 5-7, P19.
- Stavrov, S. S., and B. Kuskuley. 1993. Dependence of the iron-histidine frequency of deoxy heme proteins on the structure of its active center: quantum chemical study. In *Fifth International Conference on the Spectroscopy of Biological Molecules*. T. Theophanides, J. Anastassopoulou, and N. Fotopoulos, editors. Kluwer Academic Publishers, Norwood, MA. 305-306.
- Takano, T. 1977a. Structure of myoglobin refined at 2.0 Å resolution. I. Crystallographic refinement of metmyoglobin from sperm whale. *J. Mol. Biol.* 110:537-568.
- Takano, T. 1977b. Structure of myoglobin refined at 2.0 Å resolution. II. Structure of deoxymyoglobin from sperm whale. *J. Mol. Biol.* 110: 569-584.
- Teraoka, J., and T. Kitagawa. 1981. Structural implication of the heme-linked ionization of horseradish peroxidase probed by the Fe-histidine stretching Raman line. *J. Biol. Chem.* 256:3969-3977.
- Zerner, M., and M. Gouterman. 1966. Porphyrins. IV. Extended Hückel calculations on transition metal complexes. *Theoret. Chim. Acta.* 4: 44-63.
- Zerner, M., M. Gouterman, and H. Kobayashi. 1966. Porphyrins. VII. Extended Hückel calculation on iron complexes. *Theoret. Chim. Acta.* 6:363-400.