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Theoretical Study of the Distal-Side Steric and Electrostatic Effects on the Vibrational Characteristics of the FeCO Unit of the Carbonylheme Proteins and Their Models

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ABSTRACT The vibronic theory of activation and quantum chemical intermediate neglect of differential overlap (INDO) calculations are used to study the activation of carbon monoxide (change of the C-O bond index and force field constant) by the imidazole complex with heme in dependence on the distortion of the porphyrin ring, geometry of the CO coordination, iron-carbon and iron-imidazole distances, iron displacement out of the porphyrin plane, and presence of the charged groups in the heme environment. It is shown that the main contribution to the CO activation stems from the change in the σ donation from the 5σ CO orbital to iron, and back-bonding from the iron to the $2\pi^*$ orbital of CO. It follows from the results that none of the studied distortions can explain, by itself, the wide variation of the C-O vibrational frequency in the experimentally studied model compounds and heme proteins. To study the dependence of the properties of the FeCO unit on the presence of charged groups in the heme environment, the latter are simulated by the homogeneous electric field and point charges of different magnitude and location. The results show that charged groups can strongly affect the strength of the C-O bond and its vibrational frequency. It is found that the charges located on the distal side of the heme plane can affect the Fe-C and C-O bond indexes (and, consequently, the Fe-C and C-O vibrational frequencies), both in the same and in opposite directions, depending on their position. The theoretical results allow us to understand the peculiarities of the effect of charged groups on the properties of the FeCO unit both in heme proteins and in their model compounds.

INTRODUCTION

Heme proteins (HPs) play central roles in the life of organisms (Eichorn, 1973). The major classes of heme proteins share the same prosthetic group, heme IX, but differ in the axial coordination of the iron and the distal environment of the heme. In myoglobins (Mbs), hemoglobins (Hbs), peroxidases, and *b*- and *c*- cytochromes, imidazole (Im) is one of the iron axial ligands. In Mb and Hb the sixth coordination position is empty or weakly ligated by the solvent. The process of coordination of diatomic molecules by some kinds of HPs lies at the basis of their very important ability to store and transport them. The coordination of diatomics leads also to their activation (change of interatomic distance, force field constant, and activation barrier of dissociation). For example, cytochrome P-450 breaks the O-O bond of coordinated dioxygen (Ullrich, 1979). In this case the energy required to cleave this bond in the coordinated state decreases by one order of magnitude in comparison to free O_2.

The nature of the proximal iron ligand, hydrogen bonds, steric and electrostatic interactions of the distal heme environment with the diatomics, and the geometry of coordination of the latter are the essential factors through which the protein regulates their storage, transport, or catalytic activities. One major problem is that of discriminating between the different factors affecting these properties of HPs.

The infrared and resonance Raman spectroscopies allow us to investigate directly the effects of the proximal and distal heme environment on the activation of diatomics by HPs. These methods, applied to carbonmonoxy complexes of HPs and their models (for reviews, see Babcock, 1988; Yu and Kerr, 1988; Ray et al., 1994), showed that even in Hbs, Mbs, and their models containing a complex of carbonylheme with neutral imidazole, Fe(P)(Im)(CO), the C-O and Fe-C stretching frequencies (ν_{CO} and ν_{FeC}) vary within a very wide range, from 1932 to 2014 cm^{-1} and from 486 to 514 cm^{-1}, respectively. Several mechanisms were invoked to explain these variations of ν_{CO} and ν_{FeC}. It was assumed, first, that they can be caused by the electron donor-acceptor interactions between the proximal nitrogen of the distal histidine and the coordinated carbon monoxide (Maxwell and Caughey, 1976; Makinen et al., 1979; Fuchsman and Appleby, 1979; Ray et al., 1994). However, the distance between the distal histidine and carbon monoxide (~3 Å) seems to be too large to provide an effective electron density transfer from the former to the latter (Hanson and Schoenborn, 1981; Kuriyan et al., 1986; Cheng and Schoenborn, 1991; Cameron et al., 1993; Quillin et al., 1993, 1995; Jewsbury and Kitagawa, 1994, 1995; Li et al., 1994).

Second, the distortion of the CO coordination geometry due to the steric interaction with the distal environment was considered to be another cause of the dependence of the FeCO properties on the protein structure (Maxwell and Caughey, 1976; Case and Karplus, 1978; Makinen et al.,

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1979; Fuchsman and Appleby, 1979; Alben et al., 1982; Kerr et al., 1983; Yu et al., 1983; Campbell et al., 1987; Yu and Kerr, 1988; Li and Spiro, 1988; Desbois et al., 1989; Lee and Oldfield, 1989; Morikis et al., 1989; Ramsden and Spiro, 1989; Potter et al., 1990; Nagai et al., 1991; Balasubramanian et al., 1993; Braunstein et al., 1993; Mourant et al., 1993; Hirota et al., 1994). The data of the earlier x-ray (Kuriyan et al., 1986) and neutron diffraction (Hanson and Schoenborn, 1981; Cheng and Schoenborn, 1991) studies and molecular dynamics calculations (Case and Karplus, 1978; Kuriyan et al., 1986) supported the possibility of this distortion in HPs, but these results were questioned by the latest investigations (Cameron et al., 1993; Quillin et al., 1993, 1995; Li et al., 1994; Springer et al., 1994). All of the available data on the model compounds (Ricard et al., 1986; Kim et al., 1989; Kim and Ibers, 1991; Tetreau et al., 1994) also manifest the same linear perpendicular coordination of CO. The possibility of the Fe-C distance variation in different HPs and model compounds and the effect of this variation on the frequencies under consideration was discussed by Ray et al. (1994). Note also that in principle the porphyrin ring deformation can also affect ν_{FeC} and ν_{CO}.

Third, the electrostatic interaction between the protein polar groups and the heme is under intensive discussion now (Li and Spiro, 1988; Morikis et al., 1989; Augspurger et al., 1991; Oldfield et al., 1991; Park et al., 1991; Brantley et al., 1993; Lian et al., 1993; Cameron et al., 1993; Sakan et al., 1993; Hu et al., 1994; Ivanov et al., 1994; Li et al., 1994; Ray et al., 1994; Springer et al., 1994; Decatur and Boxer, 1995).

The intensive experimental investigation of the HPs under consideration and their models have stimulated a number of the quantum chemical calculations of Fe(P)(Im)(CO) complexes, simulating the active centers of these proteins. Zerner et al. (1966), Loew and Kirchner (1978), Case et al. (1979), Dedieu et al. (1979), and Herman et al. (1980) showed that tilting and bending of CO change the strengths of the Fe-C and C-O bonds. Paul and Rosen (1984), Smith et al. (1984), and Paul et al. (1985) showed that both ferric/ferrous reduction potential and the presence of the point charges in the heme vicinity affect the population of the antibonding $2\pi^*$ molecular orbital (MO) of the coordinated CO. These results imply that the distortions of the FeCO unit due to the charged groups close to the heme must change both ν_{FeC} and ν_{CO}. However, the magnitude of these changes was not obtained in the papers mentioned above.

The study of electric field effects on the electronic structure and adiabatic surface of the free carbon monoxide molecule showed (Augspurger et al., 1991) that polarization of this molecule must itself lead to the pronounced dependence of its ^{13}C and ^{17}O isotropic chemical shifts and C-O stretching frequency on the field. This conclusion was used by Oldfield et al. (1991) to state that in Hbs and Mbs ν_{CO} is controlled mainly by the electric field of the distal histidine. This and other (Lambert, 1984, 1988; Bauschlicher, 1985; Andrès et al., 1991; Bishop, 1993) results on the free CO molecule obviously do not take into account covalent interaction of CO with the rest of the complex, which strongly affects the strength of the C-O bond and essentially depends on the applied electric field (Paul and Rosen, 1984; Smith et al., 1984; Paul et al., 1985). Therefore, to study the effect of the heme environment on ν_{CO} in the frameworks of the traditional quantum chemical approach, the ground-state equilibrium configuration of the Fe(P)(Im)(CO) complex and the cross sections of its adiabatic surface in the direction of the corresponding normal coordinates in the presence of the protein environment or model electric field must be found. Realization of this plan for such a large transition metal complex is very difficult.

At the same time, the vibronic theory of activation (VTA) was recently developed (Bersuker, 1978, 1984). This theory elucidates the relationship between the metal-ligand orbital electron density transfers (OEDTs) and the activation of the ligand due to its coordination. In particular, VTA allows us to obtain such parameters of the coordinated ligand as its interatomic distances and force field constants without calculating its adiabatic potential, provided that the OEDTs to or from the ligand upon its coordination are known. In our previous work (Stavrov et al., 1993) we combined the VTA results with extended Hückel self-consistent charge quantum chemical calculations of OEDTs and applied this approach to the investigation of CO activation due to its coordination by HPs. In this way (Stavrov et al., 1993) we were able to describe the activation of carbon monoxide upon its binding to HPs and their models.

In this work OEDTs are calculated using a semiempirical quantum chemical technique of the intermediate neglect of differential overlap (INDO) version of MO LCAO approach, which was developed (Ridley and Zerner, 1973; Bacon and Zerner, 1979; Zerner et al., 1980; Anderson et al., 1986) and applied (Herman et al., 1980; Waleh and Loew, 1982a,b,c; Loew, 1983; Edwards et al., 1986; Du et al., 1991; Du and Loew, 1992, 1995; Harris and Loew, 1993) to study electronic structure and the spectroscopic properties of a number of heme proteins and their models.

The above-described combined VTA-INDO approach is used to study theoretically the dependence of ν_{CO} and ν_{FeC} of the FeCO unit of carbonylheme proteins and their models on the distortion of the CO coordination geometry, change of the iron-carbon and iron-imidazole distances, displacement of the iron out of the porphyrin plane, ruffling and doming of the porphyrin ring, a homogeneous electric field, and point charges located in the distal environment of the heme. In particular, the following general questions will be answered:

1) To what extent can at least one of the frequencies under consideration be affected by the heme distortions and charges mentioned above?

2) How does each of the events mentioned above affect the relative magnitudes of ν_{CO} and ν_{FeC}?

3) What kind of effects (heme distortion or charged groups) can explain the notable changes of ν_{CO} and ν_{FeC} in different HPs and their models?

Preliminary results of this study have been presented elsewhere (Kushkuley and Stavrov, 1994).

METHODS

The intermediate neglect of differential overlap (INDO) version of the MO LCAO approach is used for quantum chemical calculation of the Fe(P)(Im)(CO) electronic structure and orbital charge transfers, Δq_i. This type of approach is based on the self-consistent solution of the Hartree-Fock equation with the inclusion of all the one-center exchange terms necessary for rotational invariance and accurate spectroscopic predictions, as well as an accurate description of integrals involving 3d atomic orbitals (Ridley and Zerner, 1973; Bacon and Zerner, 1979; Zerner et al., 1980; Anderson et al., 1986). INDO is the technique in which one-center core integrals are obtained just from ionization potentials, and the ionization potentials and electron affinities are used for calculation of the ground-state electronic configuration. For calculation of the low-, intermediate-, and high-spin states of iron porphyrin complexes, the spin restricted Hartree-Fock method (RHF) was used and was shown (Waleh and Loew, 1982a,b,c; Loew, 1983; Edwards et al., 1986; Du et al., 1991; Du and Loew, 1992, 1995; Harris and Loew, 1993) to correctly describe the ground state of this system. In this work the INDO calculations were performed using Mataga-Nishimoto parameterization (Ridley and Zerner, 1973; Bacon and Zerner, 1979; Zerner et al., 1980; Anderson et al., 1986). For quantum chemical calculations by this method we used the ZINDO program (kindly supplied by Dr. M. Zerner, Department of Chemistry, University of Florida).

To calculate the OEDTs to and from CO the MOs obtained by the INDO calculations were rewritten in the basis of the eigenfunctions of the free CO and atomic functions of other atoms. Then the occupations of the CO eigenfunctions in the complex were calculated as the Mulliken population of the corresponding orbitals.

The basic structure of the Fe(P)(Im)(CO) complex is presented in Fig. 1. For the calculations the known structures of the porphyrin and imidazole rings (Eaton et al., 1978) were used. The distances Fe-N_{Im} = 2.04 Å and Fe-C = 1.745 Å were taken from the x-ray diffraction studies of the model compounds (Ricard et al., 1986; Kim et al., 1989; Kim and Ibers, 1991; Tetreau et al., 1994), and for the C-O distance the free molecule value (1.128 Å) was used (Huber and Herzberg, 1979).

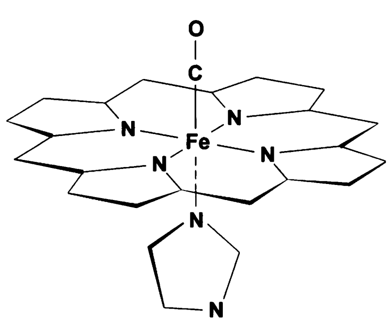

FIGURE 1 Structure of the Fe(P)(Im)(CO) complexes.

The vibronic theory of activation is described in detail elsewhere (Bersuker, 1978, 1984; Stavrov et al., 1993). Its main idea is to build a bridge between the MO description of the electronic structure and the coupling of electronic states with the nuclear configuration described by the vibronic coupling theory (Bersuker, 1978, 1984).

For this reason, like the integral linear diagonal vibronic constants,

$$F^{(I)}=\langle I|(\partial H/\partial R)_{R=R_0}|I\rangle\,, \tag{1}$$

orbital vibronic constants were introduced (Bersuker, 1978, 1984).

$$f_i=\langle i|(\partial H/\partial R)_{R=R_0}|i\rangle\,, \tag{2}$$

and it was shown that for the diagonal constants $F^{(I)}$ and f_i

$$F^{(I)} = \sum_i q_i^{(I)} f_i\,. \tag{3}$$

In these formulae, i and I are the wave functions of the one-electron ith MO and the Ith state of the diatomic, respectively; H is the Hamiltonian; R is the interatomic nuclear coordinate; R_0 is its equilibrium magnitude; $q_i^{(I)}$ is the ith MO population number in the Ith electronic state of the diatomic; and the electronic configuration of different I states differ by the excitation of the electron from the ith to the jth MO.

Similar to Eqs. 1 and 2, the expressions for the curvature constants, K and k_i, and anharmonicity constants, Γ and γ_i, can be written.

The diagonal constants $F^{(I)}$ and $f^{(i)}$ have the physical meaning of the force with which the electronic distribution in the Ith or ith state acts upon the nuclear configuration in the R direction. For a free molecule in the equilibrium position

$$F^{(I)}(Q_0) = \sum_i q_i^{(I)} f_i = 0\,. \tag{4}$$

By coordination or perturbation (excitation, ionization, reduction, etc.) q_i changes to $q_i + \Delta q_i$. In the first-order approximation with respect to Δq_i a distorting force, ΔF, and changes of curvature, ΔK, and anharmonicity, ΔG, at the equilibrium point of free CO Q_0 occur (below the index of the electronic state I is omitted for simplicity):

$$\begin{aligned} \Delta F(Q_0) &= \sum_i \Delta q_i f_i \\ \Delta K(Q_0) &= \sum_i \Delta q_i k_i \\ \Delta \Gamma(Q_0) &= \sum_i \Delta q_i \gamma_i\,. \end{aligned} \tag{5}$$

Eq. 5 is valid for any rearrangement of the electronic structure of the molecule, including excitation, ionization, reduction, and coordination, provided that the orbital electron transfers Δq_i are sufficiently small as compared to the whole valence electron charge.

The appearance of the distorting force and changes of the curvature and anharmonicity of the diatomic adiabatic curve directly affect such observable properties of the coordinated ligand as its interatomic distances and vibrational frequency. To estimate the expected changes of these molecular properties from the changes of the parameters mentioned in Eq. 5, one must describe the molecular adiabatic potential in terms of the model potential (Bersuker, 1978, 1984), $\epsilon(Q)$. In this paper the Morse potential is used to describe analytically the adiabatic potential of a diatomic:

$$\epsilon(R) = D\{1 - \exp[-\alpha(R - R_0)]\}^2\,, \tag{6}$$

where D is the dissociation energy and α is the parameter defining the curvature of the potential curve.

The constants f_i, k_i, and γ_i were considered as empirical parameters and were obtained (Stavrov et al., 1993) for the chemically active 5σ and $2\pi^*$ CO molecular orbitals (MO) from the experimental data on different electronic states of free carbon monoxide:

$$\begin{gathered} f(5\sigma) = -0.25 \text{ mdyn}, \quad k(5\sigma) = 1.23 \text{ mdyn/Å}, \\ \gamma(5\sigma) = 16.45\ mdyn/\text{Å}^2, \quad f(2\pi^*) = -1.71 \text{ mdyn}, \\ k(2\pi^*) = 4.66 \text{ mdyn/Å}, \quad \gamma(2\pi^*) = -21.04 \text{ mdyn/Å}^2. \end{gathered} \tag{7}$$

The ground-state values of $K_0 = 19.00$ mdyn/Å and $\Gamma_0 = -131.22$ mdyn/Å^2 were also used for the calculations. Note that the 5σ orbital vibronic constants are considerably smaller than the $2\pi^*$ ones. This is a result of the weak antibonding character of the 5σ MO.

Using all of these parameters and having calculated the orbital charge transfers from 5σ and to $2\pi^*$ CO MOs, $\Delta q(5\sigma)$ and $\Delta q(2\pi^*)$, we are able to estimate the above properties of the coordinated carbon monoxide.

RESULTS

To investigate the effect of charged groups and heme and CO distortion on the C-O and Fe-C bonds, the electronic structures of different Fe(P)(Im)(CO) complexes with and without external point charges (Fig. 2) were studied with the INDO-RHF approach. Note, first of all, that the neglect of the configurational interaction with single- and double-electron excited configuration is justified by the conclusion of Herman et al. (1980) and Waleh and Loew (1982a,b,c) that these configurations very weakly contribute to the electronic structure of the ground state of Fe(P)(Im)(CO). Our calculations on the electric field effect also support this conclusion.

Second, any distortion of Fe(P)(Im)(CO) can be caused only by the interaction with the protein environment. In principle, the effect of this interaction on the CO coordination geometry can be studied for each protein. However, the problem of the correct consideration of this interaction, including, for example, correct consideration of the protein electric field and choice of the protein dielectric constant, is too complicated to guarantee a high accuracy of the results.

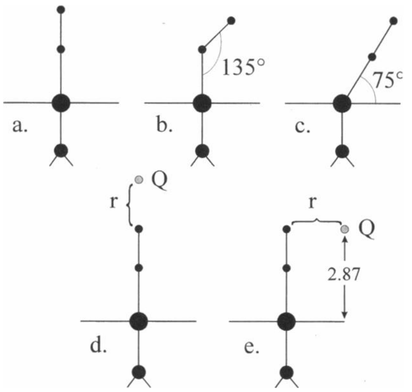

FIGURE 2 Schematic representation of the geometry of the complexes with linear perpendicular (*a*), bent (*b*), and tilt (*c*) coordinated CO without and with (*d* and *e*) point charge *Q*.

Therefore in this paper to estimate the contribution of different Fe(P)(Im)(CO) structural parameters to the control of ν_{CO} and ν_{FeC} we calculate the effect of the different distortions on the electronic structure of the compound under consideration, as it was done, for example, by Zerner et al. (1966), Loew and Kirchner (1978), Case et al. (1979), Dedieu et al. (1979), and Herman et al. (1980).

All of the calculations support our previous results (Stavrov et al., 1993), namely, that the 5σ and $2\pi^*$ CO MOs provide major contributions to the formation of the Fe-CO bond. OEDTs from the CO 3σ, 1π, and $4\sigma^*$ MOs to the iron are weak ($\Delta q(3\sigma) = -0.02\ e^-$, $\Delta q(4\sigma^*) = -0.08\ e^-$ and $\Delta q(1\pi) = 0\ e^-$) and practically do not depend on the presence of point charges or distortion of the CO coordination geometry, the only exception being the CO bending, which causes $\Delta q(1\pi) = -0.04\ e^-$. Therefore, upon calculating ν_{CO} we only take into account the effect of the change of 5σ and the $2\pi^*$ MO population. This approximation slightly overestimates ν_{CO} reduction upon coordination, but allows us to evaluate the dependence of this frequency on the structure of the compound under consideration.

To investigate the effects of the CO distortion on the properties of the FeCO unit the Fe(P)(Im) complexes with linear perpendicular-, bent-, and tilt-coordinated CO (Fig. 2, *a–c*) were studied. The calculated charges of Fe, CO and porphyrin, Fe-C and C-O bond orders, and $\Delta q(5\sigma)$ and $\Delta q(2\pi^*)$ are presented in Table 1 (because the $2\pi_x^*$ and $2\pi_y^*$ orbitals contribute equally to the CO activation, the change of the net population of both orbitals is presented in this table and below in the text). It follows from these data that $\Delta q(5\sigma)$ and $\Delta q(2\pi^*)$ for the linear tilted coordination are higher, and those for the bent one are lower than for the

TABLE 1 Effect of the CO distortion on the charge distribution in Fe(P)(Im)(CO) and the properties of FeCO unit

Coordination geometry	Iron charge (e^-)	Porphyrin charge (e^-)	CO charge (e^-)	Fe-C bond index	C-O bond index	$\Delta q(5\sigma)$ (e^-)	$\Delta q(2\pi^*)$ (e^-)	K(CO) (mdyn/Å)	ν_{CO} (cm^{-1})
Linear									
R(Fe-C) = 1.73 Å	−0.09	−0.25	−0.01	1.15	2.06	−0.34	0.45	15.76	1969
R(Fe-C) = 1.745 Å	−0.10	−0.25	0	1.12	2.07	−0.34	0.43	15.89	1977
R(Fe-C) = 1.77 Å	−0.10	−0.26	−0.02	1.08	2.10	−0.33	0.40	16.10	1990
Tilt	−0.11	−0.19	−0.04	1.06	2.01	−0.35	0.49	15.43	1949
Bent	−0.08	−0.26	−0.01	1.04	2.05	−0.28	0.42	15.95	1976

*The imidazole charge is the same for all of the structures, +0.35 e^-.

linear perpendicular coordination. Extended Hückel self-consistent charge calculations (Howell et al., 1990) performed by us to test this conclusion showed the same trend (the data are not presented). Note that in our previous paper (Stavrov et al., 1993) the OEDTs for the bent structure represented in the corresponding line of table 3 were different because of an error in the calculation.

Using Eqs. 5 and 6, and data on $\Delta q(5\sigma)$ and $\Delta q(2\pi^*)$, we also calculated the dependence of the force field constant, K(CO), of coordinated CO on its coordination geometry. Then the ν_{CO}s were calculated (Table 1) using the K constants evaluated and taking into account vibrational coupling in the FeCO unit, K(FeC) = 2.48 mdyn/Å, H(Fe-C-O) = 0.8 mdyn * Å/rad^2 and K(FeC, CO) = 0.8 mdyn/Å (Li and Spiro, 1988).

The effect of the Fe-CO distance (R_{FeC}) on the strengths of the Fe-C and C-O bonds and corresponding frequencies was studied by the calculation of the electronic structure of Fe(P)(Im)(CO) with the R_{FeC} changed in the region between 1.73 Å and 1.77 Å, which corresponds to the variation of this distance in the model compounds (Ricard et al., 1986; Kim et al., 1989; Kim and Ibers, 1991; Tetreau et al., 1994).

The calculations show that increase of the R_{FeC} markedly reduces both σ donation and π back-bonding, causing an increase in the C-O bond index (B_{CO}) and a decrease in the Fe-C bond index (B_{FeC}), the latter change being considerably stronger than that of the former (Table 1). This result implies that a change in R_{FeC} must lead to opposite changes in strengths of the C-O and Fe-C bonds and corresponding frequencies. Application of VTA shows that the 0.04 Å variation of R_{FeC} changes ν_{CO} by 21 cm^{-1}, approximately by 5 cm^{-1} for each 0.01 Å.

The effect of the displacement of the iron out of the porphyrin plane was studied by the displacement of the axial nuclear system CO-Fe-Im by 0.1 Å from the porphyrin plane in both directions with respect to the plane (x-ray studies of the model compounds by Ricard et al., 1986; Kim et al., 1989; Kim and Ibers, 1991; and Tetreau et al., 1994, show that this displacement is smaller than 0.05 Å). The distances between the iron and axial ligands were kept constant.

It was found that such a displacement changes $\Delta q(2\pi^*)$ very weakly (~0.01 e$^-$), causing a 5 cm^{-1} change in ν_{CO}, whereas B_{FeC} can be significantly affected by such a distortion, from 1.08 to 1.16. It follows from this result that the iron out-of-plane displacement can notably affect ν_{FeC} and not ν_{CO}.

To estimate the effect of the iron-imidazole distance ($R_{Fe\text{-}N(Im)}$), the electronic structure of Fe(P)(Im)(CO) was studied for two magnitudes of $R_{Fe\text{-}N(Im)}$, 2.04 Å and 2.08 Å, obtained in the x-ray studies of the model compounds (Ricard et al., 1986; Kim et al., 1989; Kim and Ibers, 1991; Tetreau et al., 1994). The results show that neither B_{CO} (ν_{CO}) nor B_{FeC} is significantly affected by the change of the Fe-N$_{Im}$ distance in the region under consideration.

Effect of the porphyrin ring deformation on ν_{CO}

Several kinds of deformations (Ricard et al., 1986; Kim et al., 1989; Kim and Ibers, 1991; Cartier et al., 1992; Tetreau et al., 1994) of the heme were studied:

a) The S_4 ruffling deformation with one pair of opposite pyrrole nitrogens 0.12 Å above the porphyrin plane and another pair 0.12 Å below the plane. The corresponding distal pyrrole carbons were displaced by 0.2 Å below and above the porphyrin plane, respectively.

b) The S_4 ruffling deformation with one pair of opposite mesocarbons 0.5 Å above the porphyrin plane and another pair 0.5 Å below the plane. The porphyrin pyrrole rings were rotated around the iron-pyrrole nitrogen bonds to keep each ring in the same plane with the corresponding mesocarbon (Tetreau et al., 1994).

c) Two doming deformations with all of the pyrrole nitrogens 0.12 Å below the porphyrin plane and the distal pyrrole carbons 0.2 Å above the same plane and vice versa.

In all of these cases iron was kept in the plane of four nitrogens and distances between the iron and the axial ligands were not changed.

The results show that none of the distortions of the porphyrin ring nuclear configuration described above affect the population of CO 5σ and $2\pi^*$ MOs. This fact, as well as the independence of the C-O bond index (B_{CO}) on the ring distortion, brings us to the conclusion that the studied ruffling and doming of the porphyrin ring do not affect ν_{CO} notably. The bond index of the Fe-C bond is not changed by the ruffling and is very slightly affected by the doming (from 1.22 to 1.21), implying that only a weak decrease of

ν_{FeC} should be caused by ring doming, whereas ruffling hardly affects this frequency.

The effect of charged groups and electric field on the FeCO unit was studied by locating different point charges, Q, in the vicinity of the Fe(P)(Im)(CO) complex with linearly coordinated CO (Fig. 2, *d* and *e*). The electronic structure of this complex in the presence of point charges $Q = \pm 0.5\ e^-$ and $\pm\ 1.0\ e^-$ located at distances r = 2.5, 3.0, 4.0, 5.0, and 7.0 Å from the CO oxygen in the direction perpendicular (Fig. 2 *d*) and parallel (at a distance of 2.87 Å from the porphyrin) to the porphyrin plane (Fig. 2 *e*) was obtained using the same INDO-RHF technique. To calculate K(CO) and ν_{CO} the above-described VTA procedure was used. It was found that the presence of a charge in the heme vicinity strongly affects electron density transfers in the Fe(P)(Im)(CO) (Fig. 3). This essentially changes B_{CO} and B_{FeC} (see Fig. 4) and, consequently, the C-O (Fig. 5) and Fe-C vibrational frequencies.

The effect of the homogeneous electric field of magnitude from −0.02 to 0.02 a.u. (1 a.u. = 5.14225 * 10^9 V/cm) oriented perpendicular and parallel to the porphyrin plane, on C-O and Fe-C bond indexes and C-O vibrational frequencies, was also studied. The results show that the perpendicular field changes ν_{CO} from 2042 to 1880 cm^{-1}, B_{CO} from 2.26 to 1.86, and B_{FeC} from 0.97 to 1.36, whereas the parallel field hardly affects the parameters under consideration.

The large variation of B_{FeC} implies that the electric field affects the strength of the Fe-C bond. As a result, the change of the length of this bond could be expected. However, the results of the calculations using the same INDO technique and theoretical values of gammas show that the field of 0.01 a.u. directed perpendicular to the porphyrin plane affects the Fe-C bond length very weakly (~0.005 Å).

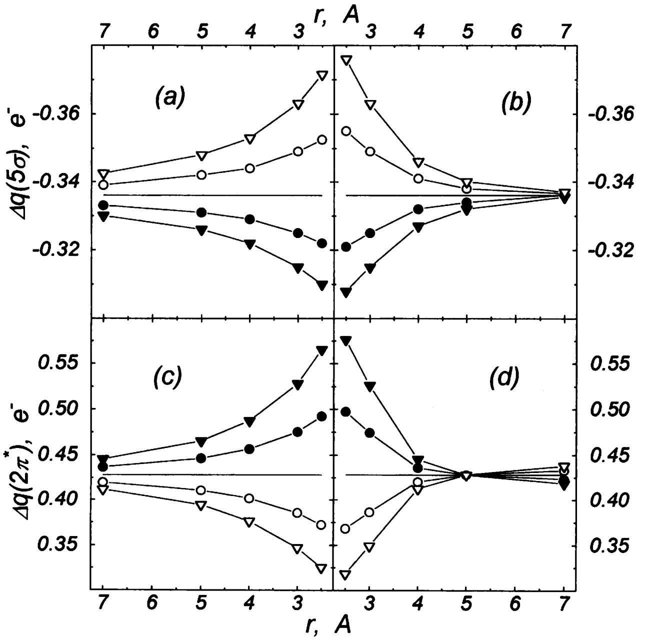

FIGURE 3 Dependence of the σ donation (*a* and *b*) and π back-bonding (*c* and *d*) on the magnitude of the point charge (+1 e^-, ▼; +0.5 e^-, ●; −0.5 e^-, ○; −1 e^-, ▽; no charge, solid line) and its displacement in the perpendicular (*a* and *c*) and parallel (*b* and *d*) directions with respect to the porphyrin plane.

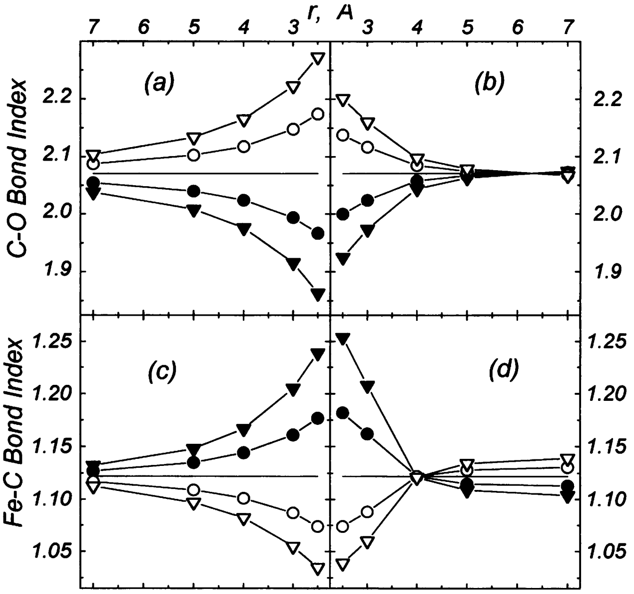

FIGURE 4 Dependence of the C-O (*a* and *b*) and Fe-C (*c* and *d*) bond indexes on the magnitude of the point charge and its displacement in the perpendicular (*a* and *c*) and parallel (*b* and *d*) directions in respect to the porphyrin plane. Notations for different charge magnitudes are the same as on Fig. 3.

To obtain the results on the relationship between the C-O and Fe-C vibrational frequencies and corresponding bond indexes, note first of all that at small changes of the force field constant K the change of the vibrational frequency ν equals

$$\Delta\nu = (\nu + \Delta\nu) - \nu = \sqrt{\frac{K}{M}}\left(\sqrt{1 + \frac{\Delta K}{K}} - 1\right) \approx \nu\frac{\Delta K}{2K}. \tag{8}$$

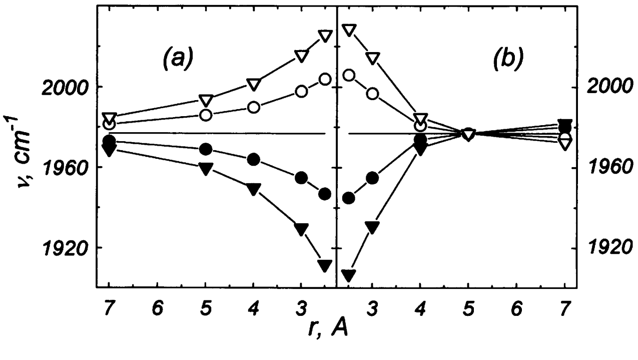

FIGURE 5 Dependence of ν_{CO} on the magnitude of the point charge and its displacement in the perpendicular (*a*) and parallel (*b*) directions in respect to the porphyrin plane. Notations for different charge magnitudes are the same as on Fig. 3.

Then, taking into account that all the perturbations of the Fe(P)(Im)(CO) system (the heme geometry changes and the charges) affect the absolute values of B_{FeC} and B_{CO} relatively weakly (up to 10%), we can state that the dependence of each bond index on the perturbation must be close to linear. Therefore, the relationship between the changes in these bond indexes (ΔB_{FeC} and ΔB_{CO}) is also expected to be close to linear. Assuming that the relative change of the force field constants is proportional to the corresponding relative change of the bond indexes, we can write for the relationship between the changes of the force field constants and bond indexes:

$$\frac{\Delta K}{K} = \zeta \frac{\Delta B}{B}, \tag{9}$$

where ζ is a coefficient of proportionality. Using Eqs. 8 and 9 we obtain for the relation between the changes of ν_{FeC} and ν_{CO}

$$\frac{\Delta \nu_{FeC}}{\Delta \nu_{CO}} = \xi \frac{\nu_{FeC}}{\nu_{CO}} \frac{B_{CO}}{B_{FeC}} \frac{\Delta B_{FeC}}{\Delta B_{CO}} \tag{10}$$

where ξ describes the possibility that the ζ coefficients for the Fe-C and C-O bonds can be different.

Using the following values of the vibrational frequencies and bond orders $\nu_{CO} \approx 2000\ \mathrm{cm}^{-1}$, $\nu_{FeC} \approx 500\ \mathrm{cm}^{-1}$, $B_{CO} \approx 2$, and $B_{FeC} \approx 1$ and notations

$$\begin{aligned} \alpha_\nu &= \Delta \nu_{FeC} / \Delta \nu_{CO} \\ \alpha_B &= \Delta B_{FeC} / \Delta B_{CO}, \end{aligned} \tag{11}$$

and assuming that $\xi \approx 1$, we obtain for the relationship between α_ν and α_B

$$\alpha_\nu \approx \tfrac{1}{2} \alpha_B . \tag{12}$$

DISCUSSION

It follows from our calculations that the major contribution to the Fe-CO bond provides a σ donation from the weakly antibonding 5σ MO of CO to the iron 4s and $3d_z{}^2$ orbitals and π back-bonding from the iron $3d_\pi$ orbitals to the strongly antibonding CO $2\pi^*$ MO. Therefore, an increase in both the σ donation and π back-bonding strengthens the Fe-C bond, whereas the C-O bond is strengthened by an increase in the σ donation and a decrease in the π back-bonding. As a result, in the cases where the back-bonding is mainly affected we should expect an opposite change in the strengths of the Fe-C and C-O bonds (Fe-C and C-O vibrational frequencies), whereas a change predominantly in the σ donation must lead to a change in the same direction in the bonds under consideration. In the intermediate case when both σ donation and π back-bonding are essentially affected by the charge, a complicated correlation between the Fe-C and C-O vibrational frequencies should be expected.

Effect of the porphyrin ring deformation, iron out-of-plane displacement, and change of the iron-imidazole distance

The porphyrin ring doming and ruffling and change of the iron-imidazole distance of reasonable magnitude do not affect K(CO) and ν_{CO} notably. Therefore, the heme distortions themselves cannot be considered a major cause of the essential changes of ν_{CO}, observed in different HPs and their models. ν_{FeC} can be weakly affected by the porphyrin ring doming. The iron out-of-plane displacement considered can weakly affect both ν_{CO} ($\geq 5\ \mathrm{cm}^{-1}$) and B_{FeC} (and, consequently, ν_{FeC}).

From these results we can conclude that the distortions under consideration cannot explain the wide variability of ν_{CO} in HPs and their models.

Effect of the CO distortion

Our results confirm the qualitative conclusion (Li and Spiro, 1988) that CO bending and tilting must affect the properties of the complex in different ways. Because the interaction between the π electrons of Fe and CO (and not of porphyrin) mainly controls the distribution of the FeCO π electrons, the CO tilt causes a tilt of the whole π electron subsystem of this unit. Such a tilt notably reduces an overlap between the iron d_π and porphyrin $e_g(\pi^*)$ orbitals and, consequently, decreases iron-porphyrin back-bonding. The main part of the liberated π electron density is transferred to the antibonding $2\pi^*$ MO of CO and the rest remains on the iron (Table 1). It is this additional electron density transfer to $2\pi^*$ MO that causes the reduction in K(CO) and makes it smaller than in the case of the linear perpendicular CO coordination (Fig. 2 *a*).

The CO bending causes overmixing of the σ- and π-electron subsystems. It decreases electron density transfer from the 5σ to the $2\pi^*$ MOs by 0.06 e^- and 0.01 e^-, respectively, and increases donation from the bonding 1π MO of CO by 0.04 e^- (Table 1). The effect of the latter OEDT was not taken into account in the K(CO) calculation because of the lack of the experimental data on the free CO excited states necessary for the estimation of the corresponding orbital vibronic constants. Therefore, the value of K(CO) for the bent CO coordination represented in the Table 1 should be considered an overestimation, and it is expected to be in the region between the magnitudes of K(CO) for linear and tilt-coordinated CO.

Support for this conclusion comes from data on the C-O bond index (Table 1). It is possible to see from the corresponding column that indeed both bending and tilting weaken this bond, the effect of the latter being much stronger.

In the last column of Table 1 are presented the magnitudes of ν_{CO} calculated taking into account the vibrational interactions between the Fe-C, C-O, and Fe-C-O oscillators. Because in the tilt and linear perpendicular complexes the geometry of the FeCO unit is the same, the vibrational coupling of these oscillators is the same and the change of ν_{CO} completely reflects the change in K(CO). In the bent FeCO unit the vibrational repulsion of the Fe-C and C-O oscillators is weaker than in the linear one (Li and Spiro, 1988; Desbois et al., 1989), leading to additional reduction of ν_{CO}. Therefore we can conclude that both bending and tilting of CO should reduce the C-O frequency; the effect of the latter distortion is expected to be stronger.

As was mentioned above, reduction of K(CO) associated with CO tilt is caused by an increase in the back-bonding, and the reduction associated with CO bending is caused by OEDT from the 1π orbital of CO. Both electron density transfers must strengthen the Fe-C bond. However, it is seen from Table 1 that both types of distortion of linear perpendicular CO coordination geometry reduce both B_{CO} and B_{FeC} and, consequently, weaken these bonds. This relaxation of the Fe-C bond is caused by weakening of the σ bonds between CO and iron, which predominates over the strengthening of the corresponding π bonds and leads to the weakening of the net Fe-C bond (see also, Loew and Kirchner, 1978; Herman et al., 1980). Such a behavior of the Fe-C bond is to be expected, because the linear-perpendicular geometry of CO coordination corresponds to the strongest interaction between the Fe(P) and CO (Dedieu et al., 1979), any distortion being caused by the interaction with the distal environment of the porphyrin (Ricard et al., 1986; Kim et al., 1989; Kim and Ibers, 1991; Stryer, 1988; Tetreau et al., 1994). As a result, a distortion of the linear-perpendicular coordination geometry has to weaken both the C-O and Fe-C bonds (see also, Herman et al., 1980).

Effect of the Fe-C distance variation

The results obtained show that even a small increase in R_{FeC} significantly reduces back-bonding, decreasing the strength of the Fe-C bond and increasing that of the C-O bond (Table 1). Calculation of ν_{CO} shows that it also depends on R_{FeC} significantly, being changed by approximately 5 cm^{-1} by the 0.01 Å change of R_{FeC}. Using the available data on the iron-carbon force field constant, $K(FeC) = 2.48$ mdyn/Å^2 (Li and Spiro, 1988), it is easy to show within the harmonic approximation, $E = ½kq^2$, that the 0.01 Å change of R_{FeC} requires only about 0.02 kcal/mol. This implies that compression of the Fe-C bond can take place in HPs and their models and can considerably affect ν_{CO}.

It is important to note, however, that even the absolute magnitude of the change in B_{FeC} by this displacement is considerably stronger than that of B_{CO} (Table 1). As far as relative changes are considered, it follows from the data represented in the Table 1 that this Fe-C distance variation changes B_{FeC} by 6%, whereas B_{CO} is changed by only 2%. The theoretical data on the relationship between B_{FeC} and B_{CO} are represented in Fig. 6 *a* (*asterisks*), from which it is possible to see that the relationship between these two parameters is very close to the linear one with the slope $\alpha_B = -1.72$. This result is understandable, because the change in R_{FeC}, as any other change of the heme structure, has to affect first of all the Fe-C bond, the change of the C-O being considerably weaker. The dependence of the changes in ν_{FeC} and ν_{CO} on the change of R_{FeC} can be estimated using Eq. 11: $\alpha_\nu = -0.86$ ($\xi = 1$).

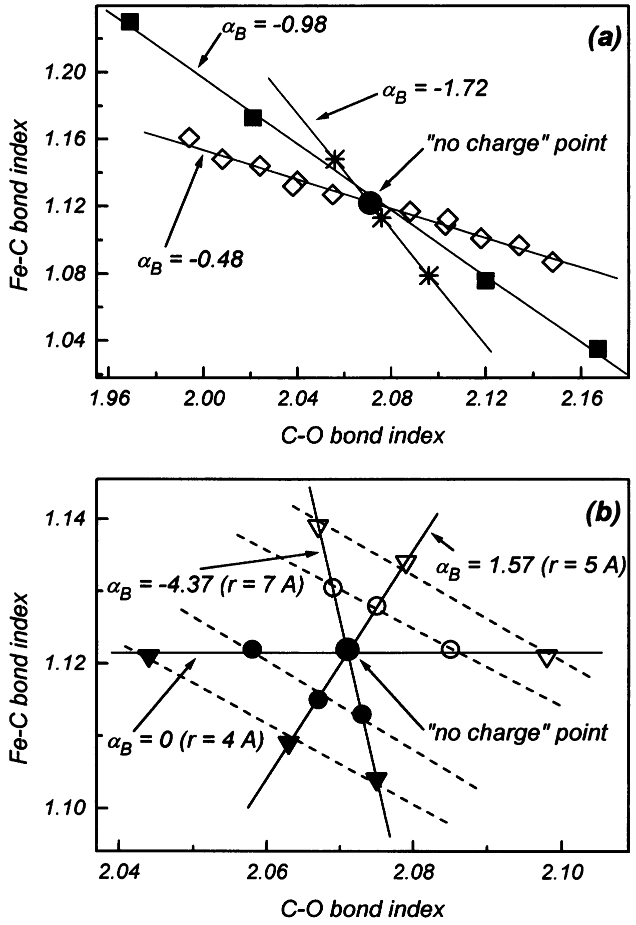

FIGURE 6 Theoretical data on the relationship between the Fe-C and C-O bond indexes in dependence on the iron-carbon distance (*a*, *), homogeneous electric field (*a*, ■) and different charges in the axial (*a*, ◇) and nonaxial positions (*b*, +1 e^-, ▼; +0.5 e^-, ●; −0.5 e^-, ○; and −1 e^-, ▽). Relationships between B_{FeC} and B_{CO} on the substitution for different charges at the same position (*b*, *solid lines*) and for the same charge at different positions (*dashed lines*) are represented.

Effect of charges and electric field on the C-O bond strength and vibrational frequency

Obviously the presence of point charges in the vicinity of CO should affect the difference in electrostatic potentials between CO and rest of the molecule. Changes in this difference should also change the electron density distribution in the FeCO unit. For example, the appearance of a positive charge in the CO vicinity should attract electron density to CO decreasing σ donation and increasing back-bonding, whereas a negative charge would be expected to affect the electron density distribution in the opposite direction (Li and Spiro, 1988; Ray et al., 1994).

This qualitative conclusion is strongly supported by our results on the influence of a point charge on the population of the CO 5σ and $2\pi^*$ MOs. Fig. 3, *a* and *c*, shows that the charge located at any distance from the CO oxygen along the FeCO bond (Fig. 2 *d*) affects the back-bonding much more strongly than the σ donation. This result implies that charged particles located along the FeCO direction are expected to affect the FeCO properties mainly through change of the iron back-bonding.

The data on the respective bond indexes are presented in Fig. 4 *a*. They show that indeed the charged particles should essentially affect the strength of the C-O bond. For example, coordination of carbon monoxide itself reduces B_{CO} from 2.48 to 2.07, whereas the appearance of a unit positive or negative charge in 2.5 Å from the CO oxygen varies this index from 1.83 to 2.34, respectively. ν_{CO} (Fig. 5) also can be strongly affected by the charged particle: coordination reduces this frequency from 2170 to 1977 cm^{-1}, whereas the presence of the same charge changes it from 1910 to 2030 cm^{-1}.

Figs. 3–5 show that displacement of the charge from CO in the direction parallel to the porphyrin plane (Fig. 2 *e*) reduces the charge effects much more strongly than that along the porphyrin axial axe. To understand the causes of this difference let us consider, for example, the effect of a positive charge. Because the charge located near the CO oxygen is closer to CO than to the iron and porphyrin, it attracts electron density to CO more strongly than to the iron and the porphyrin ring.

Displacement of the charge in the direction perpendicular to the porphyrin plane conserves this hierarchy of distances (see Fig. 2 *d*); the greater the displacement, the smaller the difference in potential between CO and iron and porphyrin. As a result we find a smooth decrease in the charge effect with its displacement (see left sides of Figs. 2–5).

Displacement in the direction parallel to the porphyrin plane, first of all, decreases the difference between the *Q*-Fe and *Q*-CO distances, causing a faster decrease in the potential difference between iron and CO. Second, such a displacement does not affect the *Q*-porphyrin distance (Fig. 2 *e*) and, consequently, does not practically affect the porphyrin potential. Therefore, attraction of the electron density to the porphyrin is not practically affected by the charge displacement, whereas its attraction to CO decreases even faster than in the previous case. Combination of these two mechanisms causes a faster decrease in the charge effects in the case of the parallel charge displacement. Note that because π electrons are more polarizable than the σ ones, dependence of the back-bonding on the charge coordinate must be much sharper than the dependence of the σ donation (Fig. 3, *b* and *c*).

For example, a positive charge located at 5 Å from the FeCO line still reduces σ donation but does not practically affect back-bonding. Because both B_{CO} and ν_{CO} are controlled mainly by $\Delta q(2\pi^*)$, the effect of such a charge on the B_{CO} (Fig. 4 *b*) and ν_{CO} (Fig. 5 b) is negligible. The charge located at $r > 5$ Å already attracts π electrons to the porphyrin ring more strongly than to CO and, as a result, reduces the back-bonding in respect to its "no charge" value, the effect of this charge on the σ donation being very weak (Fig. 3, *b* and *c*). Therefore, a positive charge located at a distance greater than 5 Å is expected to strengthen the C-O bond and increase respective vibrational frequency. It implies, for example, that the charges of opposite signs located closer and farther away than 5 Å from the FeCO line must cause changes in the same direction of $\Delta q(2\pi^*)$ and, consequently, of ν_{CO}.

The homogeneous electric field of the magnitude in the range between −0.02 and 0.02 a.u. affects mainly back-bonding ($\Delta q(2\pi^*)$ changes from 0.29 to 0.63), the σ donation being affected much more weakly ($\Delta q(5\sigma)$ changes from −0.33 to −0.36). These variations in OEDTs change B_{CO} by 0.4 (from 1.86 to 2.26) and ν_{CO} by 160 cm^{-1} (from 1880 to 2042 cm^{-1}), the magnitude of these changes depending linearly on of the field variation. Note that the same variation in the electric field affects ν_{CO} of the free carbon monoxide about 2 times more weakly (Augspurger et al., 1991).

Note that the effect of the point charges and electric field on the geometry of Fe(P)(Im)(CO) is ignored in this study. This assumption is based on the result that ν_{CO} is almost unaffected by all of the distortions of the complex under consideration, except the change in the Fe-C distance. The latter was shown to be changed very weakly by the applied electric field (~0.005 Å at the field of 0.01 a.u.). Therefore, neglect of this change is not expected to cause uncertainty in ν_{CO} larger than 5 cm^{-1} (see Table 1). To understand qualitatively the cause of such a weak field effect on the Fe-C distance in spite of the strong field dependence of B_{FeC}, let us consider for simplicity the effect of the positive electric field. Such a field causes electron density transfer to CO, which on the one hand increases the strength of the Fe-C bond and on the other increases the CO negative and iron positive charges. Therefore, the direct interaction of the field under consideration with the Fe-CO unit tends to extend the Fe-C bond, whereas the electron density transfer tends to shorten it. Our calculations show that the latter effect is slightly stronger than the former one. Because the reduction of the Fe-C distance increases ν_{CO}, the neglect of its relaxation leads to the slight (<5 cm^{-1}) underestimation of the field effect on this frequency.

Effects of charges and electric field on the relationship between Fe-C and C-O bond strengths and frequencies

It has been noted already that any displacement of the charge in the direction perpendicular to the porphyrin plane along the Fe-CO line affects $\Delta q(2\pi^*)$ much more strongly than $\Delta q(5\sigma)$; the smaller the charge magnitude and the more distant the charge, the closer are these OEDTs to the "no charge" value (Fig. 3, *a* and *c*). Thus, as it was mentioned earlier in this section, appearance of a charge in the region under consideration must cause opposite changes of C-O and Fe-C bond strengths (Fig. 4, *a* and *c*) and corresponding vibrational frequencies. Moreover, because in this case the charge is closer to CO than to any other part of the heme complex, it affects absolute magnitudes of the C-O bond strength more strongly than that of the Fe-C bond (Fig. 4, *a* and *c*), contrary to the situation of the heme distortion.

A different situation occurs for the charges located relatively close to the porphyrin plane (in our case, at a distance of 2.87 Å from this plane). Indeed, the dependence of $\Delta q(2\pi^*)$ on the charge coordinate crosses the "no charge" line at $r = 5$ Å. The $\Delta q(5\sigma)$ depends less on the charge presence and reaches its "no charge" value at $r \approx 7$ Å (Fig. 3, *b* and *d*). Thus, a charge located in the region of 5 Å affects $\Delta q(5\sigma)$ more strongly than $\Delta q(2\pi^*)$ (Fig. 3, *b* and *d*). Because a change in the σ donation affects the strengths of the Fe-C and C-O bonds in the same direction, the charge located in this region should also affect these bonds (and their frequencies) in the same direction; this conclusion is supported by the results of our calculations (Fig. 4, *b* and *d*). The charges located at $r > 7$ Å again affect the $\Delta q(2\pi^*)$ more strongly than $\Delta q(5\sigma)$, causing opposite changes in B_{CO} and B_{FeC}.

Note also that the charges located close to the heme plane strongly interact with the porphyrin and essentially affect redistribution of the electron density between the porphyrin and the FeCO unit. Consequently, dependence of the Fe-C bond strengths on the charge position in this case must be much sharper than in the case of the axially located charges (compare Fig. 4, *c* and *d*). Moreover, the relative dependence of B_{FeC} on the charge coordinate is stronger than that of B_{CO} (Fig. 4 *d*). This fact makes possible situations in which the charge affects B_{CO} and does not practically affect B_{FeC}, and vice versa (Fig. 4, *b* and *d*; $r = 4$ Å and 5–7 Å region, respectively).

As a result, we can conclude that the appearance of charges relatively close to the porphyrin plane at $r = 4–7$ Å must lead to a very complicated relationship between the changes of the Fe-C and C-O bond strengths and, consequently, corresponding frequencies.

Taking into account that $r = 5$ Å approximately corresponds to the edge of the porphyrin ring, we can conclude from this discussion that the charges located relatively close to the porphyrin plane and porphyrin ring edge would affect the relationship between B_{CO} and B_{FeC} in a very complicated manner. At the same time, the charges located close to the FeCO line (or far enough from the porphyrin plane) would reverse the strengths of the bonds under consideration.

The relationship between B_{CO} and B_{FeC} for the axially located distal charges and homogeneous electric field studied in this work is presented in Fig. 6 *a*. The charges of different magnitude located along the Fe-CO line at different distances from the CO oxygen alter the C-O and Fe-C bond indexes in opposite directions, because these charges affect mainly the back-bonding. The correlation between B_{FeC} and B_{CO} is very close to the linear one, with $\alpha_B = -0.48$. For the same reason, the electric field also affects these bond indexes in the opposite direction, the only difference being in the higher α_B (−0.98). The origin of this difference is well understood, because the charges are located closer to the CO than to the rest of the complex, affecting the C-O bond more strongly than the Fe-C one.

The charges located 2.87 Å from the porphyrin plane and closer than 4 Å from the Fe-CO line also alter B_{CO} and B_{FeC} in opposite directions, the correlation also being almost linear, with $\alpha_B = -0.78$. However, the effect on this relationship of the charges located in the region between 4 Å and 7 Å from the porphyrin axis is very complicated (Fig. 6 *b*), because of the very complicated relationship between the changes in $\Delta q(2\pi^*)$ and $\Delta q(5\sigma)$ caused by them. It can be seen from Fig. 6 *b* that the appearance of the charges in this region can change both B_{FeC} and B_{CO}, leading to the very different slopes of the relationship between these bond indexes. All of the substitutions at 4 Å from the axis of the complex under consideration have almost no affect on B_{FeC} ($\alpha_B = 0$), the substitutions at 5 Å cause a change in B_{FeC} and B_{CO} in the same direction ($\alpha_B = 1.57$), and the appearance of the charges at 7 Å leads to an opposite change in B_{FeC} and B_{CO} ($\alpha_B = -4.37$). At the same time, as it follows from the same data (Fig. 6 *b*), a change in the location of each of the charges leads to the opposite change in B_{FeC} and B_{CO}, the relationship between them being close to linear, with slopes between −0.54 and −0.62.

From Eq. 11 and theoretical data on α_B, and assuming $\xi = 1$, we can easily deduce that the appearance of different axially located distal charges must lead to a linear relationship between changes in the frequencies under consideration, with a slope $\alpha_\nu \approx -0.24$; the homogeneous electric field would lead to $\alpha_\nu \approx -0.5$, whereas the charges located close to the porphyrin plane and CO (not in the region of the porphyrin edge) must cause almost linearly interrelated changes of these frequencies with $\alpha_\nu \approx -0.38$. Appearance of different charges near the porphyrin edge (4–7 Å from the porphyrin center) should lead to a very complicated relationship between ν_{FeC} and ν_{CO}, whereas a change in their locations in the direction parallel to the porphyrin plane is expected to lead to $\alpha_\nu \approx -0.3$.

This implies that a substitution of a nonpolar group for a polar one in the region of the porphyrin edge should cause a very complicated relationship between ν_{FeC} and ν_{CO}, whereas a change in the position of this group (for example, in the series of the analogous compounds) is expected to cause opposite changes in these frequencies. Both the substitution of the nonpolar group for a polar one in the region of the porphyrin edge and a change in its location in the series of analogous compounds is expected to lead to the linear relationship between ν_{FeC} and ν_{CO} with $\alpha_\nu \approx -0.3$, the whole linear dependence being shifted up or down with respect to the "no charge" point (compare to Fig. 6 *b*).

Comparison with the experimental data

Let us consider, first, model carbonyl complexes with neutral imidazole, the experimental data for which are presented in Fig. 7. The available x-ray diffraction studies show that in these compounds the CO coordination geometry is very close to the linear perpendicular one (Ricard et al., 1986; Kim et al., 1989; Kim and Ibers, 1991; Tetreau et al., 1994), and, consequently, the wide variation of ν_{CO} in the compounds under consideration cannot be explained in principle by the ligand distortion. It follows also from our calculations that none of the investigated distortions of the porphyrin ring can significantly affect ν_{CO}.

The only variation of the heme complex geometry that can affect both ν_{FeC} and ν_{CO} is a change in R_{FeC}, but it is impossible to explain from this point of view the change of ν_{CO} in a number of model compounds (Kim et al., 1989; Kim and Ibers, 1991). For example, a change in the distal environment of α-PocPiv(NMeIm) with respect to C_2-Cap-(NMeIm) has almost no affect on ν_{FeC} (500 and 497 cm^{-1}), but ν_{CO} is affected very strongly (1964 cm^{-1} and 2002 cm^{-1}, respectively). Moreover, a change in R_{FeC} in these two compounds (1.74–1.75 Å in the former and 1.77 Å in the latter) itself is expected to cause an opposite change in ν_{CO}. The much stronger change in ν_{CO} than in ν_{FeC} ($\alpha_\nu = -0.38$; Fig. 7, *solid circles*) takes place in the series of the strapped heme complexes FeSP-n ($n = 13$, 14, and 17 and describes the length of the aliphatic chain providing distal steric hindrance). This relationship also cannot be explained by taking into account only the effect of R_{FeC} alteration.

The only other interaction that can explain the very wide distribution of ν_{CO} in the model compounds is an electrostatic interaction between the heme and charged groups attached to the different substituents at the porphyrin ring. In general, among all the considered perturbations only distal charges could cause weaker a change in B_{FeC} than in B_{CO} and, consequently, only these charges are expected to be able to cause the experimentally observed (Fig. 7), considerably weaker change in ν_{FeC} than in ν_{CO}.

In many model compounds the charged or electron-rich groups are located close to the porphyrin edge in the region of 4 Å $< r <$ 7 Å (for a review, see Momenteau and Reed, 1994). In these compounds different charged or polar substituents must cause the complicated relationship between the C-O and Fe-C vibrational frequencies. Additional contribution to the scattering of ν_{FeC} can stem from the variation, even very weak, of R_{FeC}, distortion of the porphyrin ring, or the iron out-of-plane displacement. Thus, the model complexes with different charged or polar substituents are expected to demonstrate the poor correlation between ν_{FeC} and ν_{CO}, with strong scattering of the corresponding points around the "no charge" point. At the same time, in the series of the model compounds with the same charged or polar substituents located at different points in the space close to the porphyrin edge, the graph of the dependence between ν_{FeC} and ν_{CO} must be close to a line that does not pass through the "no charge" point and has a slope $\alpha_\nu \approx -0.3$. Therefore, in the case of the model compounds the charged groups themselves must lead to a complicated relationship between ν_{FeC} and ν_{CO}, which consists of the superposition of the scattered points around the "no charge" point and a number of almost linear relationships, with $\alpha_\nu \approx -0.3$ not crossing the "no charge" point.

Indeed, for the relation between the experimental values of ν_{FeC} and ν_{CO} of all the model compounds (Fig. 6 *b*) we obtain a very low correlation coefficient of linear regression, $R = -0.63$. The whole figure represents a superposition of the linear plots with $\alpha_\nu = -0.38$ and -2.28, corresponding to SP-n and Piv_2C_n series, respectively, and the points scattered around the "no charge" point with coordinates of $\nu_{FeC} \approx 495$ cm^{-1}, $\nu_{CO} \approx 1965$ cm^{-1} (the theoretical value $\nu_{CO} = 1977$ cm^{-1} was calculated for the "no charge" situation at $R_{FeC} = 1.745$ Å).

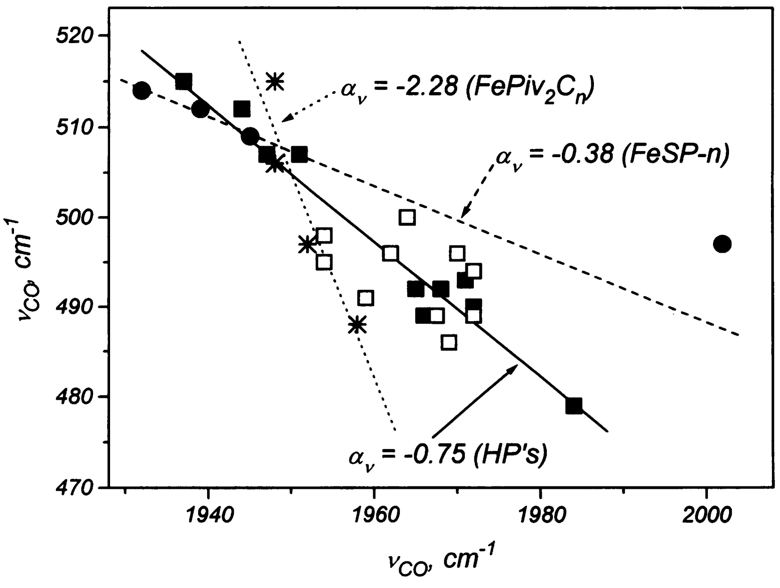

FIGURE 7 The experimental data (Ray et al., 1994) on the C-O and Fe-C vibrational frequencies for the heme proteins (■), SP-n (●), and Piv_2C_n (*) and other (□) model carbonyl complexes with neutral imidazole.

The relationship between ν_{FeC} and ν_{CO} in the SP-n series is very close to the theoretically expected one ($\alpha_\nu \approx 0.3$) for the case of the charged or polar substituents located close to the porphyrin edge at slightly different distances from the axis of the porphyrin ring. This implies that in the series under consideration a change in the length of the aliphatic chain causes mainly a change in the location of charged substituents.

It is impossible, however, to explain from the same point of view the very large value of $\alpha_\nu = -2.28$ in the carbonyl complexes of a series of the superstructured iron-porphyrins with the general formula Fe(II)(Piv)$_2$C$_n$ (Desbois et al., 1989; Tetreau et al., 1994). In this formula *n* describes the length of the aliphatic chain providing distal steric hindrance and equals the number of CH$_2$ groups in this chain ($n = 6, 7, 8, 10$). The only other perturbation that can notably affect both ν_{FeC} and ν_{CO} is a change in the iron-carbon distance. Indeed, x-ray diffraction study of the compounds with $n = 6$ and 8 showed that a decrease in the length of the chain causes a decrease in R_{FeC} from 1.752 ± 0.004 Å to 1.733 ± 0.004 Å, and changes the porphyrin ring doming and ruffling and positions of the polar groups of the aliphatic chain. This change of the complex structure increases ν_{FeC} from 497 to 515 cm^{-1}, whereas ν_{CO} decreases much less markedly, from 1952 to 1948 cm^{-1}, and in general a decrease in *n* from 10 to 6 increases ν_{FeC} from 488 to 515 cm^{-1} and decreases ν_{CO} from 1958 to 1948 cm^{-1}. Our calculations show that an increase in R_{FeC} from 1.733 to 1.752 Å itself must increase ν_{CO} by 10 cm^{-1}, a considerably stronger effect than the experimentally observed 4 cm^{-1}. This discrepancy may result from an error in the x-ray diffraction studies of the compounds under consideration. The possibility of such an explanation stems from the precision of the R_{FeC} evaluation (± 0.004 Å), the precision for ΔR_{FeC} being, consequently, on the order $\Delta R_{FeC} = 0.019 \pm 0.008$ Å. From the latter it could be concluded that the actual change in R_{FeC} is smaller than, or on the order of, 0.01 Å, which should cause a change in ν_{CO} of 5 cm^{-1}. It was mentioned above, however, that for this kind of change in the heme geometry the theoretical value of α_ν is expected to be close to -0.9, whereas the experimental value of α_ν is -2.28. (Fig. 6 *b, asterisks*). This pronounced difference between the theoretical and experimental values of α_ν implies that there is an additional interaction that affects ν_{FeC} much more strongly than ν_{CO}. Because the change of the porphyrin ring geometry does not affect ν_{FeC} so strongly, the effect of the charged groups located in the region of the porphyrin edge ($r = 5$–6 Å) is the only possible explanation of the large value of α_ν. Indeed, in these compounds there are charged NH groups located close to the porphyrin edge that provide positive polarity. It follows from Fig. 6 *b* that when the positive charges approach the porphyrin center with the reduction in length of the aliphatic chain B_{FeC} increases and B_{CO} decreases, amplifying the increase in α_B (and, consequently, in α_ν) due to the reduction in R_{FeC} upon this chain shortening. This joint effect of the change of R_{FeC} and position of the charged groups naturally explains the large negative value of α_ν.

The location of the FeC$_2$-Cap(NMeIm) point ($\nu_{FeC} = 497$ cm^{-1}, $\nu_{CO} = 2002$ cm^{-1}) close to the line corresponding to the FeSP-n compounds can be explained as a result of the electrostatic interaction with the charged groups of the four chains linking the benzene to the porphyrin that are located close to the porphyrin edge and, therefore, affect ν_{CO} much more strongly than ν_{FeC} (see Fig. 5, *b* and *d*). The change in R_{FeC} can also essentially affect ν_{FeC} and ν_{CO} of this compound.

As in the case of the model compounds, the recent data on HPs (Cameron et al., 1993; Quillin et al., 1993, 1995; Li et al., 1994; Springer et al., 1994; Lim et al., 1995) also show that the heme environment does not cause essential distortion of the CO coordination geometry. Consequently, electrostatic effect of the heme environment must be invoked to explain the wide variation in ν_{CO} in the proteins. The specificity of the situation in the heme proteins stems from the fact that it is very difficult to realize a point mutation close to the plane of the porphyrin ring 4–7 Å from its center. All other substitutions, with respect to protein differences, affect back-bonding more strongly than the σ donation. This implies that the relationship between the Fe-C and C-O vibrational frequencies in different HPs is expected to be closer to the inverse linear dependence than that in the model compounds (compare to Fig. 7). Note that the experimental points corresponding to HPs form a line with slope $\alpha_\nu = -0.75$, which is three times larger than the value of -0.24 obtained for the effect of distal charges. This fact implies that the difference between ν_{CO} and ν_{FeC} in different HPs stems not only from the different distal environment, but also from the different proximal environments (theoretical value for this case, $\alpha_\nu \approx -0.55$; unpublished result) or different structures of the whole proteins, which produce different electric fields close to the homogeneous one (theoretical value of $\alpha_\nu = -0.5$). Additional contributions to α_ν can stem from the differences in R_{FeC} produced by different heme environments.

The weak bending or tilting of CO can also modulate the relationship between ν_{FeC} and ν_{CO}, but cannot by themselves cause opposite changes in these frequencies.

It also follows from our results that the charge located on the distal side of the heme affects notably not only the closest C-O and Fe-C bonds but also the Fe-N$_{Im}$ bond up to ±10%, the Fe-N$_{Im}$ and Fe-C bond indexes being altered in the same direction. This implies that charged groups located on the distal side of the heme must lead to a significant change in strengths of both the Fe-N$_{Im}$ and Fe-C bonds in the same direction.

Effect of the environment on the heme electronic structure and its spectroscopic manifestations

The traditional interpretation (Stryer, 1988) of the experimental data has been based on three main assumptions: 1) the dependence of a diatomic (CO or O_2) affinity on the protein type is controlled by different geometries of the ligand coordination in these proteins; 2) these different coordination geometries are caused by different "steric" interactions with the distal environment in different proteins; 3) the "steric" interactions are described usually by the Lenard-Jones potential (Atkins, 1986) and, consequently, are supposed to affect, first of all, the nuclear configuration of the complex (geometry of the CO coordination), the electronic subsystem being affected through the change of the nuclear one. However, all of the interactions between any two molecules are caused by different types of noncovalent electrostatic (charge-charge, dipole-charge, dipole-dipole, dipole-induced-dipole, etc.) and dispersion (interelectronic) interactions (Atkins, 1986). All of the electrostatic interactions must affect both the electronic and nuclear subsystems. Moreover, because electrons are lighter and move faster than the nucleus, the electron subsystem is expected to be affected by this interaction more strongly than the nuclear one (for example, the dispersion interaction is caused only by the electron subsystem) (Atkins, 1986).

The only exception can be the case where the electrons and nuclei interact very strongly and electrons cannot move freely from one atom to another under the external field, as in the case of localized electrons in organic compounds. In this case any change of the shape of the electron cloud strongly affects the nucleus positions and vice versa. Therefore in this case we really can suppose that the essential part of the intermolecular interactions can be described as a result of the change of the nuclear configuration or, in other words, the "steric" interaction prevails.

It follows from our theoretical study that the π electron subsystem of Fe(P)(Im)(CO) is very sensitive to the presence of charges, and any electrostatic interaction should first of all affect the π electron distribution. If we agree with this result and with the statement that the intermolecular repulsion or attraction is caused by some electrostatic interactions, we should conclude that the interaction between Fe(P)(Im)(CO) and the heme environment would affect most strongly the π electron distribution, the geometry of the heme complex being less affected due to the distortion of the π electron cloud and nonbonding "steric" interactions. Therefore, to discuss any spectroscopic manifestation of the CO coordination geometry distortion caused by the heme environment, we must take into account not only the manifestations of the distortion itself but also the direct effect of the electrostatic interaction with the heme environment, which causes this distortion (see also, Oldfield et al., 1991; Park et al., 1991; Ray et al. 1994).

It is the redistribution of the π electrons that changes the Fe-C and C-O bond orders in opposite directions, leading to the experimentally observed opposite change of the Fe-C and C-O frequencies in different heme proteins.

CONCLUSION

Application of the vibronic theory of activation and INDO quantum chemical calculations to the study of the activation of CO by different heme proteins and their models showed that a major contribution to the CO activation arises from the electron density transfer from the 5σ orbital of CO to the heme (σ donation) and from the heme to the $2\pi^*$ orbital of CO (back-bonding).

Investigation of the effect of the different distortions of the porphyrin ring showed that ruffling and doming of the porphyrin ring, displacement of iron out of the porphyrin plane, and change in the iron-imidazole distance cause very weak changes in the C-O vibrational frequency and, therefore, cannot in principle explain by themselves the wide range of the C-O vibrational frequency in different heme proteins and their models.

Distortion of the carbon monoxide linear perpendicular coordination geometry or change in the iron-carbon distance affects both σ donation and back-bonding and, in principle, can notably affect both Fe-C and C-O vibrational frequencies. However, by themselves these changes of the heme geometry can explain neither the wide range of ν_{CO} nor the inverse relationships between ν_{FeC} and ν_{CO} in the model compounds.

Appearance of the charges in the vicinity of the heme affects both the back-bonding and σ donation and can very strongly affect activation of the coordinated CO, causing a change in the C-O bond index up to ±50% of its change due to the coordination and changing the C-O vibrational frequency up to ±30% of its change upon coordination. The Fe-C and Fe-N_{Im} bonds can be also essentially affected (up to ±10%) by the distal charges.

Axially located distal charges affect mainly back-bonding and, consequently, change the Fe-C and C-O bond strengths and corresponding vibrational frequencies in opposite directions. The charges located in the region of the porphyrin edge affect both σ donation and back-bonding and lead to the very complicated relationship between ν_{FeC} and ν_{CO}. The presence of these charges explains the relationships between these frequencies in the model compounds, whereas in the heme proteins the relationship between the frequencies under consideration can be explained by the changes on the proximal side of the heme or by the substitution or/and displacement of different charged groups located far enough from the porphyrin ring.

All of the results obtained for the dependence of the Fe-C bond strength unequivocally show that the protein charged groups can essentially affect the affinity of different heme proteins to carbon monoxide and other diatomics.

In general, these theoretical results strongly support the experimental observations (Li and Spiro, 1988; Park et al., 1991; Brantley et al., 1993; Lian et al., 1993; Cameron et al., 1993; Ivanov et al., 1994; Li et al., 1994; Ray et al., 1994; Springer et al., 1994) that charged or polar amino acid groups can control both spectroscopic and thermodynamic properties of HPs, essentially affecting the properties of the

Fe-CO unit. Moreover, because the charge effect decreases slowly upon the charge displacement away from the heme, we can expect that charged groups located even relatively far from the heme can notably affect the affinity of HPs for different ligands and different spectroscopic properties of these proteins. Note that in this paper the effect of only the model point charges and homogeneous electric field on the extent of the changes of ν_{CO} and ν_{FeC} was studied. The effect of the specific point mutations on ν_{CO}, which is of special interest from this point of view, will be studied in detail in our future works.

Note also that some of our other results (the paper under preparation) on the effect of the point charges located on the imidazole side of Fe(P)(Im)(CO) show that these charges can also essentially affect the properties of the active center under consideration.

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