

Shape of the carbon monoxide infrared absorption band of carboxyheme proteins as a probe of the protein anharmonicity

Solomon S. Stavrov

*Sackler Institute of Molecular Medicine, Department of Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Tel Aviv, 69978, Israel
Tel.: +972(3) 640 9859; Fax: +972(3) 640 5168; E-mail: stavrov@post.tau.ac.il*

Abstract. Theory of the CO infrared absorption band of carbonmonoxyheme proteins is developed using results of the theory of optical absorption bandshape of impurity center in crystal. It is shown that the bandshape is controlled by electrostatic interaction to the polar or/and charged heme surrounding. Analysis of the CO bands of different heme proteins brings us to conclusion, that the CO band is broadened by very slow ($\tau > 10$ ps) motions of the heme surrounding and this motion most probably corresponds to the slow collective motion of the protein molecule. Therefore the second moment of the band must depend linearly on temperature at $T > 25$ K if the heme surrounding moves harmonically. The motion of the protein formed surrounding of the heme is arrested by the glassy protein environment. It is shown that Gaussian is the only possible symmetric shape of the CO band, if the heme surrounding moves harmonically. Deviation from this bandshape is a manifestation of anharmonic character of the surrounding motion. In general, CO infrared absorption band is shown to be an excellent probe of the dynamics of the heme surrounding.

Keywords: Protein dynamics, myoglobin, glycerol, heme proteins

1. Introduction

Dynamics of biological molecules are studied very intensively by virtually all methods of modern chemistry and physics. Special role in these studies play heme proteins (HPs), because their active center, heme, can be studied by virtually all spectroscopic techniques. In particular, it was kinetic measurements of CO recombination after photolysis of carbonmonoxy myoglobin (Mb(CO)) that lead to the view of conformational substates (CSS) and energy barriers [2,3,9,10]. Population of CSSs is very important for the protein function and leads to strongly anharmonic motion of proteins [14]. Therefore, study of the transformation of the protein motion from the harmonic to anharmonic and dependence of this transformation on the protein structure, temperature of the sample and its state (liquid or solid) is studied very intensively, see, for example, [7,10,30].

Infrared absorption (IR) spectra of carbon monoxide molecule coordinated by the heme iron of carbonmonoxyheme proteins, HP(CO), were intensively used to study structure and dynamics [2,6,15–17, 21,28,33,35,36] of different HP(CO)s. This band corresponds to transition between the ground and first

excited vibrational states of a normal vibration, which has the major contribution of the C–O valence vibration of the coordinated CO molecule. The band is well isolated from other protein IR bands, its position varying from 1900 through 2000 cm^{-1} in different HP(CO)s. In some of them the band consists of several components, each of which is usually assigned to different CSSs of the protein. Modern techniques provide one with reliable information not only on position of the CO band, but also on its shape, see, for example, [2,16,17,28,36]. The shift in the CO band position is caused by electrostatic interaction with the heme surrounding (HS), whereas the HS motion causes the band's broadening [13,16,18–21, 25].

Contribution of different interactions into shape of a vibrational band was studied in a number of publications [1,4,5,11,12,22,24,29,34]. First, resonant transfer of vibrational energy between identical molecules causes exciton-like broadening of the band. However, in the case of active centers buried in proteins, this interaction is negligibly weak. Second, there is natural broadening, which stems from decay of excited vibrational state. In carbonyl complexes of transition metals this broadening is less than 0.2 cm^{-1} even at room temperature [27] and, consequently, hardly contributes to the shape of the CO band of HP(CO), full width at half maximum (Γ) of which is larger [2,8,16,17,20] than 10 cm^{-1} . Third, energy levels of a molecule fluctuate adiabatically due to changes in the interaction of the molecule with its surroundings; its resulting fluctuations manifest itself in the band broadening. This interaction, which sometimes is called pure dephasing, mainly contributes to the CO bandshape [16,17,25–27,36].

In this paper we develop theory of the CO bandshape and its temperature dependence assuming that the heme surrounding moves harmonically. The results are used to interpret the experimental data and to reveal at which temperatures and in what protein environment the harmonic motion of the HS transforms into the anharmonic one. It is shown that the CO band is a reliable probe of this transition.

2. Interaction of the CO vibration and the heme surrounding

Previously we showed [18,19,36] that broadening of the CO band is controlled by electrostatic interaction of moving charged or/and polar parts of HS. This interaction was shown to be relatively weak: 1 Å shift of a unit point charge changes the CO frequency by the order of 10 cm^{-1} . This implies that there must be weak coupling between the C–O stretching coordinate and the HS motion.

If both the heme-CO and HS move harmonically, this coupling can be described by the Hamiltonian

$$\begin{aligned}
 H &= H_0 + H_{\text{int}}, \\
 H_0 &= -\frac{\hbar^2}{2M} \frac{\partial^2}{\partial Q^2} - \sum_i \frac{\hbar^2}{2m_i} \frac{\partial^2}{\partial q_i^2} + \frac{1}{2} M \Omega_0^2 Q^2 + \frac{1}{2} \sum_i m_i \omega_i^2 q_i^2, \\
 H_{\text{int}} &= M \Omega_0^2 Q^2 \sum_i \alpha_i q_i,
 \end{aligned} \tag{1}$$

where H_0 is Hamiltonian of a number of non-interacting oscillators; M and m_i , Ω and ω_i , and Q and q_i , are moments, reduced masses, frequencies, and displacements along the normal coordinates consisting mainly of the C–O distance and the HS modes, respectively. H_{int} describes the coupling between these subsystems, and α_i accounts for its strength.

Since the CO subsystem is much faster than the HS subsystem, the adiabatic approximation [23,31,32] can be used to find the eigenvalues and eigenfunctions of H (1)

$$E_{N,\{n_i\}} = \hbar\Omega_0\left(N + \frac{1}{2}\right) + \sum_i \hbar\omega_i\left(n_i + \frac{1}{2}\right) - \frac{1}{2}\hbar\Omega_0\xi\left(N + \frac{1}{2}\right)^2, \quad (2)$$

$$\Psi_{N,\{n_i\}}(Q, q) = \Phi_N(Q, q_1, \dots, q_i, \dots, q_j) \prod_{n_i} \varphi_{n_i}(q_i - q_{iN}),$$

where

$$q_{iN} = -\frac{\xi_i}{\alpha_i}\left(N + \frac{1}{2}\right), \quad \xi_i = \frac{\hbar\Omega_0\alpha_i^2}{m_i\omega_i^2}, \quad \xi = \sum_i \xi_i, \quad (3)$$

and N and n_i are vibrational quantum numbers of the CO and HS subsystems, respectively.

3. Shape of the CO band

Even at relatively high temperatures excited states of the CO subsystem are hardly populated, because the energy gap between the ground and first excited state of this subsystem is about 2000 cm^{-1} . Therefore, only $0, n \rightarrow 1, n'$ transitions notably contribute to the CO band.

Probabilities of the $0, n \rightarrow 1, n'$ transitions are calculated in the dipole approximation. Using Condon approximation [23,32], neglecting dispersion of the frequencies of the HS subsystem and summing over all the $0, n \rightarrow 1, n'$ transitions one obtains [23,31,32]

$$F_{0 \rightarrow 1}(\Omega) \sim \exp\left[-S \coth\left(\frac{\beta_0}{2}\right)\right] \sum_p \exp\left(p\frac{\beta_0}{2}\right) I_p\left[\frac{S}{\sinh(\beta_0/2)}\right] \delta[\Omega - \Omega_0(1 - \xi) - p\omega_0], \quad (4)$$

where I_p is the modified Bessel function of the first kind, S is Huang–Rhys constant

$$S = \frac{1}{2} \frac{\Omega_0}{\omega_0} \xi, \quad \beta_0 = \frac{\hbar\omega_0}{k_B T}, \quad (5)$$

k_B is Boltzmann constant, and T is the sample temperature.

The natural broadening of the excited vibrational state of the CO subsystem can be taken into account [31,32] by substituting δ function in (4) for Lorentzians centered at $\Omega_0(1 - \xi) - p\omega_0$.

It is convenient to describe shape of the band using method of moments [23,32]

$$M_0 = \int_{-\infty}^{\infty} F(\Omega) d\Omega, \quad M_1 = \frac{1}{M_0} \int_{-\infty}^{\infty} F(\Omega)\Omega d\Omega, \quad (6)$$

$$M_{n(n>1)} = \frac{1}{M_0} \int_{-\infty}^{\infty} F(\Omega)(\Omega - M_1)^n d\Omega,$$

which can be calculated analytically even in the case of strong dispersion. It was shown [23,32], that in the case under consideration M_0 and M_1 (which are intensity of the band and its center of gravity,

respectively) are temperature independent, whereas M_2 (which is proportional to Γ^2) has very specific temperature dependence

$$M_2 = M_{20} + \frac{1}{2}\Omega_0 \sum_i \xi_i \omega_i \coth\left(\frac{\beta_i}{2}\right), \quad (7)$$

where M_{20} accounts for the temperature independent inhomogeneous broadening of the band [23,32].

4. Discussion

It follows from the previous section, that broadening of the band is caused by presence of a number of the $n_i \rightarrow n'_i$ transitions; the more such transitions essentially contribute to the band, the wider band is. Hence, $\omega_i < \Gamma$. In Mb(CO) even at room temperature $\Gamma \approx 10 \text{ cm}^{-1}$ [2]; consequently, $\omega_i < 10 \text{ cm}^{-1}$ and characteristic time of the HS motion $\tau > 3 \text{ ps}$. Since the band is formed by more than two $n \rightarrow n'$ transitions, one can reliably state that $\tau > 10 \text{ ps}$.

This result, first of all, strongly justifies use of the adiabatic approximation to find eigenvectors and eigenfunctions of Hamiltonian (1), because Ω_0 ($\sim 2000 \text{ cm}^{-1}$) is much larger than ω_i ($< 10 \text{ cm}^{-1}$).

Second, τ is much longer, than characteristic times of internal vibrations of amino acids. Consequently these vibrations do not contribute to the broadening. Thus, the CO band is broadened by electrostatic interaction of the CO oscillator with some slowly moving parts of HS. If the heme pocket is not populated by solvent molecules (which can move slowly in the pocket), most probably these are motions of big parts of the protein, these parts participate in slow collective motion (SCM) of the protein molecule.

A plausible candidate for such a motion can be shift of the E helix in respect to the F helix. Indeed, in Mb(CO) histidine 64 (distal histidine) is polar and is a part of the E helix, whereas heme is covalently bonded to the F helix through histidine 93. Motion of the E helix with respect to the F helix would change the distance between the distal histidine and the heme, changing the histidine electric field on the heme-CO complex, affecting its electronic structure, and, as a result, broadening the CO band.

This picture corroborate with our previous conclusion [18,19,36] that the CO band can be broadened only by motions, which include large (in respect to the heme) displacements of charged or polar residues. It is clear, that these motions are expected to be much slower than internal vibrations of amino acids.

Third, because of such small values of ω_i , criterion $\beta_i/2 \ll 1$ fulfils at $T > 25 \text{ K}$ and for these temperatures expression (7) can be rewritten

$$M_2 = M_{20} + \Omega_0 \xi k_B T. \quad (8)$$

However, experimental studies of myoglobin [2], Mb(CO), and hemoglobin [8], Hb(CO), at pH ~ 7 in glycerol/water solvents undergoing glass-liquid transition at $T_c \approx 160\text{--}200 \text{ K}$ showed that M_2 linearly depends on T only in the liquid environment at $T > T_c$ and is constant at $T < T_c$, Fig. 1.

To understand cause of this deviation from the linearity we have to analyze assumptions made in the second and third sections: (a) describing interaction between the CO and HS subsystems we included in H_{int} (1) linear in q terms and neglected the higher order terms; (b) we used Condon approximation; and (c) we assumed that HS moves harmonically.

If any one of the first two assumptions was wrong, one would observe non-linear dependence of M_2 on temperature: the higher is the temperature, the stronger is non-linearity [23,31,32]. The experimental

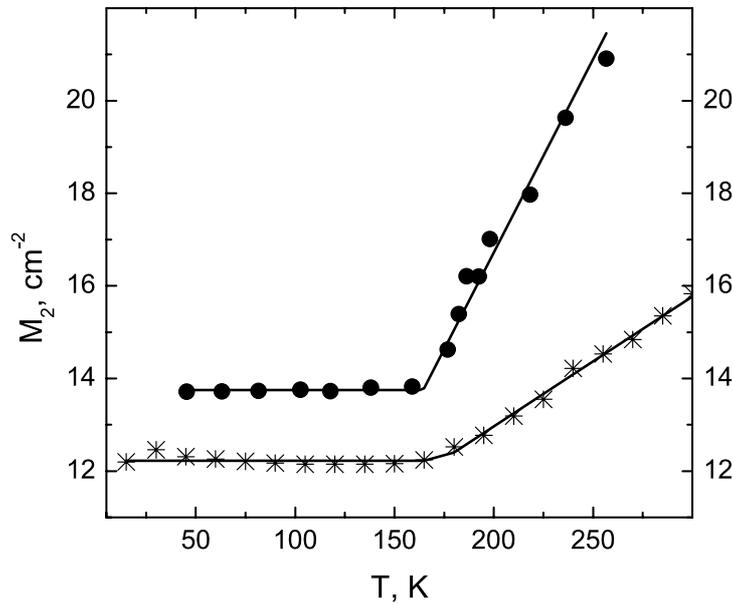


Fig. 1. Experimentally observed temperature dependences of the M_2 of different HP(CO)s (symbols) in glycerol/water solvents and their fits (lines) using (9): Mb(CO) [2] at pH 6.8 (circles, $M_{20} = 0$, $\Omega_0 \xi k_B = 0.12 \text{ cm}^{-1}$, $T_c = 165 \text{ K}$) and Hb(CO) [8] at pH 6.8 (asterisks, $M_{20} = 0$, $\Omega_0 \xi k_B = 0.12 \text{ cm}^{-1}$, $T_c = 165 \text{ K}$).

data show the totally different temperature dependence: M_2 depends linearly on T at temperatures $T > T_c$ (strongly supporting use of these approximations), whereas at lower temperatures it is constant.

Consequently, we conclude that the assumption (c) of harmonic motion of HS in the whole interval of temperatures was wrong: at $T > T_c$ HS moves harmonically, whereas at $T < T_c$ the motions, which broaden the CO band, are frozen. This can happen if the protein motions contributing into the CO broadening are arrested by the glassy environment. This explanation seems to be reasonable, because it is natural to assume, that SCMs of the protein globule (in particular, relative motions of two helices) have to be sensitive to the protein environment.

Taking into account (8), this conclusion can be formulated mathematically as follows

$$\begin{aligned}
 M_2 &= M_{20} + \Omega_0 \xi k_B T_{\text{eff}}, \\
 T_{\text{eff}} &= T_c, \quad T < T_c, \\
 T_{\text{eff}} &= T, \quad T \geq T_c,
 \end{aligned} \tag{9}$$

this expression allows to interpret the experimental data quantitatively, see Fig. 1.

Analysis of bandshape (4) showed [23,31,32] that upon heating the shape of the band becomes more symmetric and if the temperature is high enough the bandshape becomes Gaussian.

In principle, effect of natural broadening of each component of the band could transform the bandshape into Voigtian, which is a convolution of Gaussian and Lorentzian. However, the natural width [27] of each line is less than 0.2 cm^{-1} , which is much less, than total width of the band $\Gamma \geq 10 \text{ cm}^{-1}$. Consequently, the CO band can have the only possible symmetric shape – Gaussian. Considerable deviation of a symmetric CO bandshape from Gaussian (see, for example, [28]) can be a manifestation of thermal population of higher-energy CSSs, which transforms the protein motion in the anharmonic one.

5. Conclusion

We presented above theory of shape of the isolated infrared absorption band, which corresponds to the absorption of a higher-frequency oscillator linearly coupled to a number of low-frequency oscillators. We showed that this problem is analogical to the problem of optical absorption spectrum of impurity center.

The results allowed to make a number of conclusions for the case of HP(CO)s:

- (a) The CO infrared absorption band is broadened by very slow ($\tau > 10$ ps) motions of HS.
- (b) In the case of harmonic protein motion M_2 of this band must depend on temperature linearly at $T > 25$ K.
- (c) This HS motion, most probably, is a component of protein SCMs.
- (d) Transition liquid \rightarrow glass arrests SCMs, this arrest is manifested in the independence of M_2 on temperature at $T < T_c$.
- (e) Gaussian is the only possible symmetric shape of the CO band. Therefore observation of non-Gaussian IR absorption band (Voigtian, for example) hints at the population of a number of the protein CSSs and anharmonic character of the protein motion. To clarify the situation the temperature dependence experiments are necessary.

The results obtained in this paper are applicable to the complexes with other ligands like NO and O₂. Simplicity of the theoretical interpretation of this band makes it an excellent probe of the HS dynamics.

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