

## Vibrational relaxation of carbon monoxide in model heme compounds. 6-coordinate metalloporphyrins (M = Fe, Ru, Os)

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### Abstract

Vibrational relaxation of carbon monoxide bound to a series of metalloporphyrin complexes (M–(coproporphyrinate-1-tetraisopropyl ester)(CO)(pyridine); M = Fe, Ru, Os) was measured using picosecond infrared pump–probe experiments. The vibrational relaxation rates ( $(1–2.5) \times 10^{10} \text{ s}^{-1}$ ) increased with increasing mass of the metal ion. This effect is opposite that predicted by through- $\sigma$ -bond models and is interpreted as arising from a through- $\pi$ -bond coupling between the CO vibrational fundamental and porphyrin vibrations.

### 1. Introduction

Recently picosecond mid-infrared pump–probe experiments have been used to measure the vibrational lifetimes of carbon monoxide bound to heme proteins and heme model compounds [1–4]. Vibrational relaxation (VR) of carbonyl stretching vibrations of metal carbonyl compounds in liquid solution has been studied by several groups over the last few years [1–9]. The heme–carbonyl system is fundamentally different from previously studied metal carbonyls because the heme ligand is large enough to provide a bath of intramolecular states at the fre-

quency of the CO stretching excitation ( $\omega \approx 2000 \text{ cm}^{-1}$ ) [1,4,10]. In contrast to the solvent-assisted VR processes which dominate in systems such as  $\text{W}(\text{CO})_6$  [4,11], the VR of the CO stretch in heme model compounds or in heme proteins has the possibility of being a purely intramolecular process [1,4].

The CO–heme system has several other interesting features. (1) The vibrational lifetime of CO in heme proteins, where the prosthetic group is a proto-heme (i.e. iron protoporphyrinate-IX), evidences almost no temperature dependence in the 10–300 K range [1]. The lack of a significant temperature dependence shows the predominant VR mechanism does not involve direct coupling from the carbonyl stretch to the bath vibrations of frequencies  $\leq 500$

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$\text{cm}^{-1}$  [1], ruling out the possibility that VR involves phonon-assisted processes. (Here by phonons we refer to the lower frequency continuum of protein or solvent instantaneous normal modes [1,12,13].) (2) The VR lifetime of CO is sensitive to the protein structure [1–4]. Different proteins containing protoheme–CO, such as myoglobin (Mb) and hemoglobin (Hb), have different VR lifetimes [2,3]. Different conformational states of a single protein, Mb–CO, have different VR lifetimes [1]. (3) The carbonyl stretching frequency is sensitive to the heme protein structure. In protoheme and similar sterically unprotected Fe–porphyrins, the carbonyl stretch has a higher frequency, typically 1970–1980  $\text{cm}^{-1}$ . Various native and genetically engineered heme proteins can induce a carbonyl frequency redshift of tens of  $\text{cm}^{-1}$ , and the carbonyl stretch frequencies in these systems typically range from 1940 to 1980  $\text{cm}^{-1}$  [14,15]. (4) A roughly linear relation was observed between the magnitudes of the induced carbonyl frequency shifts and the VR rates in protoheme and heme proteins. The VR rate increases as the carbonyl stretching frequency decreases. An induced shift of a few tens of  $\text{cm}^{-1}$  can cause the VR rate to double [1]. (5) Two different Fe–porphyrins, protoheme and Fe tetra-*p*-sulfonatophenylporphyrin, have quite different VR rates [1]. In these two systems, the porphyrin ligands differ only in the substituents on the perimeter of the macrocycle distant from the CO binding site.

The model which is ordinarily used for VR lifetimes in condensed phase molecular systems is based on anharmonic coupling [11,16]. In this model, anharmonic interactions permit a higher energy vibration to relax by spontaneous emission of two or more lower energy excitations, which could be vibrations, phonons, or a combination of both. Vibrational lifetimes and the magnitudes of anharmonic coupling matrix elements have been calculated for a few systems such as benzene and naphthalene [17], using model atom–atom potential surfaces. These calculations have not provided much physical insight into the important problem of understanding the relationships between molecular structures and VR processes. The lack of physical insight results from the complicated and abstract relationship [16] between the atom–atom potential and the anharmonic coupling matrix elements.

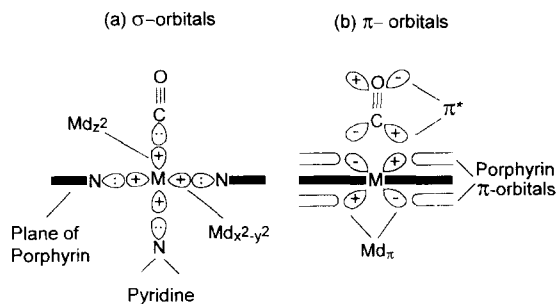


Fig. 1. Schematic of some orbitals involved in CO binding to metalloporphyrins. (a)  $\sigma$  orbitals couple the carbonyl stretch to displacements of M. For clarity the (–) lobes of  $Md_{z^2}$  and  $Md_{x^2-y^2}$  orbitals in the porphyrin plane are not shown. (b) The  $Md_{\pi}$  orbitals couple the carbonyl stretch to porphyrin  $\pi$  orbitals. Increasing back-bonding between  $Md_{\pi}$  and carbonyl  $\pi^*$  orbitals lowers the carbonyl stretching frequency and strengthens interactions between  $Md_{\pi}$  and porphyrin  $\pi$  orbitals.

In heme–CO, a simplification exists which provides a great deal of insight into VR. The vibration excited by the picosecond mid-infrared pulse is localized on the carbonyl group, and the only covalent bond between the carbonyl group and the porphyrin ligand is the Fe–CO bond. In carbonyl vibrational relaxation, anharmonic coupling should be interpreted to mean that carbonyl stretching can induce displacements of other atoms of the system. If we first consider only  $\sigma$  bonds, as shown in Fig. 1a, the most efficient through- $\sigma$ -bond VR process in heme–CO is expected to occur via coupling between the carbonyl stretch and modes which involve displacements of Fe, such as Fe–CO stretching or bending motions [1–3]. Displacements of Fe could then permit transfer of mechanical excitation from CO to vibrations of the porphyrin ligand. A quantum mechanical treatment of this scheme, which models the VR of a diatomic molecule A–B covalently bound to a third atom M which is coupled to a harmonic bath, has been discussed by Benjamin and Reinhardt [18]. They point out the importance of the mass of the atom M in determining the VR rate of the diatomic molecule A–B. For example, the far slower VR of hydroxyl groups of Si–OH versus C–OH [19] has been explained in the framework of this model as a consequence of the greater mass of the Si atom [18,19].

The through- $\sigma$ -bond mechanism for carbonyl VR in heme–CO is consistent with the observed temper-

ature dependence, because the Fe–CO bending and stretching vibrations of Mb–CO have frequencies above  $500\text{ cm}^{-1}$ , namely  $577\text{ cm}^{-1}$  (bend) and  $512\text{ cm}^{-1}$  (stretch) [15]. But other experimental observations discussed above appear inconsistent with this mechanism: First, protein structure should have little effect on anharmonic through- $\sigma$ -bond coupling between CO and porphyrin. Second, it is difficult to account for the dependence of VR rate on carbonyl stretching frequency, where a frequency change of a few percent can cause an effect of a factor of two on the VR rate. Finally, Owrutsky et al. [3,4] have noted that carbonyl stretching lifetimes in heme systems are considerably shorter than most measured in other metalcarboxyls [5,6], and have suggested the carbonyl-stretch-induced motions of Fe may not be great enough to account for the  $\approx 20\text{ ps}$  VR lifetimes observed in heme proteins.

In order to investigate the mechanisms of the VR process, we have synthesized a series of model heme compounds containing different metal ions M (M = Fe, Ru, Os) with quite different masses (56, 101 and 190 amu, respectively). The amplitudes of motion of the heavier metal ions should be much smaller than the lighter ions, so the heavier metal systems should greatly reduce the through- $\sigma$ -bond anharmonic coupling between CO and porphyrin.

## 2. Experimental

Three compounds were synthesized, M-(coproporphyrinate-I tetraisopropyl ester)(CO)(pyridine) (M = Fe, Ru, Os), hereafter denoted M(copro)(CO)(py), using established procedures. The free-base porphyrin starting material coproporphyrin-I tetraisopropyl ester, denoted  $\text{H}_2\text{copro}$ , was purchased from Aldrich Chemical. The Ru and Os compounds were prepared by metallation of  $\text{H}_2\text{copro}$  using metal carbonyl precursors  $\text{Ru}_3(\text{CO})_{12}$  and  $\text{Os}_3(\text{CO})_{12}$  under reflux to form M(copro)(CO)( $\text{H}_2\text{O}$ ) [20,21]. After metallation, pyridine ligation was provided by recrystallization from pyridine/1-hexene. Both the Ru and Os porphyrins are quite stable. They were dissolved in  $\text{CH}_2\text{Cl}_2$  solution and loaded into the optical cells. The Fe(copro)(CO)(py) is a less stable compound, and a different procedure was used. Metallation of  $\text{H}_2\text{copro}$  with  $\text{FeCl}_2$  in reflux-

ing dimethylformamide, and subsequent workup in methylene chloride/dilute HCl wash resulted in the formation of  $\text{Fe}^{\text{III}}(\text{copro})(\text{Cl})$  [22]. The  $\text{Fe}^{\text{III}}$  porphyrin was dissolved in  $\text{CH}_2\text{Cl}_2$  and was then reduced to  $\text{Fe}^{\text{II}}$  with sodium dithionite under a CO atmosphere.  $\text{Fe}(\text{copro})(\text{CO})(\text{py})$  was formed by adding an appropriate excess of pyridine.

Mid-infrared picosecond measurements were made as described previously [1,7–9], using the pump–probe apparatus (pulse duration  $t_p \approx 1.5\text{ ps}$ ) at the Stanford Free Electron Laser Center. Samples of M(copro)(CO)(py) in  $\text{CH}_2\text{Cl}_2$  solution ( $\approx 20\text{ mM}$ ) were held in 0.4 mm path length optical cells with  $\text{CaF}_2$  windows. The porphyrin concentration used gave an optical absorbance of roughly unity at the peak of carbonyl mid-infrared absorption. A Nicolet model 750 FTIR spectrometer was used to measure the carbonyl stretch spectrum.

## 3. Results

Pump–probe decays, which are interpreted to yield the vibrational lifetime of the carbonyl stretch [1–6], are shown in Fig. 2. The magnitudes of peak absorbance changes induced by pump pulses was about 0.04. All three decay curves were accurately fit by single exponentials. The VR lifetimes and carbonyl stretch frequencies are given in Table 1.

The frequency dependence of the VR rates for the three model compounds are plotted in Fig. 3. Also plotted are the rates of two different conformational substates of Mb–CO, taken from Ref. [1]. This fig-

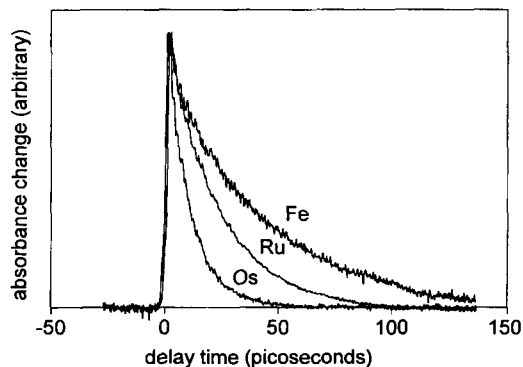


Fig. 2. Picosecond mid-infrared pump–probe data on M(copro)(CO)(py) (M = Fe, Ru, Os) at  $25^\circ\text{C}$ .

Table 1  
Vibrational lifetimes of carbonyl stretch of M(copro)(CO)(py) in  $\text{CH}_2\text{Cl}_2$  solution at ambient temperature

Metal M	Carbonyl stretch frequency	Vibrational lifetime
Fe	$1963 \text{ cm}^{-1}$	$42 \pm 2 \text{ ps}$
Ru	$1935 \text{ cm}^{-1}$	$23 \pm 2 \text{ ps}$
Os	$1902 \text{ cm}^{-1}$	$11 \pm 2 \text{ ps}$

ure shows the VR rates of the model compounds increase with decreasing carbonyl frequency, and that the rate of increase is comparable to that observed in heme proteins.

#### 4. Discussion

Our results rule out the possibility that the dominant VR channel in heme–CO systems involves through- $\sigma$ -bond anharmonic coupling between CO and heme. Table 1 shows the VR lifetime *decreases with increasing mass of the metal ion M*, which is exactly the opposite behavior predicted by the through- $\sigma$ -bond model.

In related work, we have studied the VR of  $^{13}\text{CO}$  bound to Mb [23]. Substituting  $^{13}\text{CO}$  for  $^{12}\text{CO}$  lowers the carbonyl stretching frequency from  $1945 \text{ cm}^{-1}$  to  $1902 \text{ cm}^{-1}$ , but has no effect on the VR lifetime. The carbonyl frequency redshift due to isotopic substitution is about the same magnitude as the redshift induced by substituting Os for Fe, but the origins of

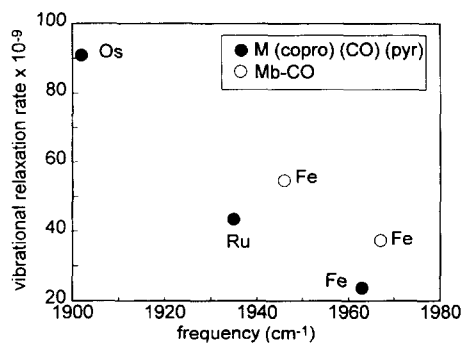


Fig. 3. Plot of carbonyl vibrational relaxation (VR) rate constants versus carbonyl stretching frequency for model heme compounds (●), and for two different conformational substates of myoglobin–CO (○).

these two frequency shifts are completely different. The isotopic shift is purely a mass effect, whereas the metal-dependent shifts are due to the effects of different metals on the electronic structures of the carbonyl and porphyrin groups. Because there is no isotope effect on the VR lifetime, this experiment shows the VR lifetime *does not depend on the absolute value of the carbonyl stretching frequency*, but instead it is correlated with the *magnitudes of induced frequency shifts caused by the differing electronic structures* of different metal ions [24,25], or by external influences such as the electric fields generated by different protein conformations [26,27].

A successful model for the VR process in heme–CO systems needs to explain the observed relationship between increasing carbonyl stretching frequency redshifts and increasing VR rates. In a quantum oscillator, the frequency can be shifted by changing either the reduced mass or the magnitudes of the interactions between masses. Our experimental result using  $^{13}\text{CO}$ , which changes the effective mass but not the force constants for the CO or Fe–CO bonds, shows we must consider effects caused by changes in interaction rather than changes in mass.

It is well known that the induced frequency redshifts of the stretching frequencies of carbonyls bound to  $d^6$ -metalloporphyrins are determined largely by the magnitude of back donation from the metal's  $d\pi$  orbitals (i.e.  $d_{xz}$  and  $d_{yz}$ ) to the carbonyl's  $\pi^*$  antibonding orbitals, termed back-bonding [15]. Fig. 3 shows the relation between decreasing carbonyl stretching frequency and increasing VR rate is observed in two quite different cases. In the first, the extent of back-bonding is determined by the increasing size of the d orbitals in the series Fe, Ru, Os. In the second, the extent of back-bonding with a single metal ion, Fe, is affected by an external influence, namely different conformations of the Mb protein.

As shown in Fig. 1b, back-bonding involves electron donation *from* the metal's  $d\pi$  orbitals *to* antibonding  $\pi^*$  orbitals of CO [28]. Increased back-bonding therefore weakens the  $\text{C}\equiv\text{O}$  bond and lowers the CO stretch frequency. Increased back-bonding also strengthens the M–CO bond [15,28]. More important for this discussion is that the  $d\pi$  metal orbitals responsible for back-bonding overlap significantly with the  $\pi$  orbitals of the porphyrin. Thus it is quite reasonable to believe that mechanical displace-

ments of the carbonyl oscillator are coupled to small-amplitude displacements of atoms of the porphyrin ligand, and this coupling increases with increasing back-bonding. We will term this mechanism through- $\pi$ -bond anharmonic coupling. Through- $\pi$ -bond coupling between axial ligands and porphyrin vibrations has been demonstrated in a number of experimental studies which have explored the effects of different axial ligands on the resonance Raman spectra of porphyrin ring vibrations [29].

Consistent with the discussion in Owrutsky et al. [3,4], the key feature of this proposed through- $\pi$ -bond mechanism is that VR of the carbonyl stretch does not arise from displacement of the M ion, but rather from dynamical changes of the effective charges of CO during vibrational motion. Because the coupling between carbonyl and porphyrin involves delocalized  $\pi$ -electron clouds, the range of interaction is much greater than the range of nearest-neighbor through- $\sigma$ -bond mechanisms proposed previously. The through- $\pi$ -bond model has several intriguing features. First, the amount of back-bonding, and presumably the VR lifetime could be significantly affected by changing the local electric fields. This may explain the different VR lifetimes of different Mb–CO conformational substates and the different VR rates of Mb–CO with point mutations produced by genetic engineering [23]. Second, changes in the proximal ligation should affect VR, since proximal ligands of different polarities and  $\pi$  polarizabilities affect the extent of back-bonding [28,29]. Finally, since the electronic structure of porphyrin can be significantly affected by the nature of substituents on the porphyrin perimeter, it is likely that molecular structural changes quite distant from the Fe–CO linkage could have a noticeable effect on VR.

In summary, the rates of CO vibrational relaxation in a series of model heme compounds increases with increasing mass of the  $d^6$ -metal ion, and increases with the extent of back-bonding from the metal's  $d$  orbitals to CO. These results cannot be explained by through- $\sigma$ -bond models where carbonyl stretching motions are coupled to the bath of porphyrin vibrations by interactions with the central metal ion. Instead we propose a through- $\pi$ -bond mechanism that involves a longer-ranged coupling between the carbonyl stretch and porphyrin vibrational states, transmitted via interactions of the  $d\pi$

orbitals of the metal ion with the  $\pi$  electrons of the porphyrin ligand. Through- $\pi$ -bond models are needed to explain carbonyl VR in metalloporphyrins because the carbonyl stretch interacts with a large aromatic porphyrin ligand. In the presence of the extended  $\pi$ -bonded porphyrin macrocycle, through- $\pi$ -bond interactions can efficiently couple the carbonyl stretch to displacements of porphyrin atoms which may be distant from the M–CO moiety. This work shows it is possible for through- $\pi$ -bond anharmonic coupling mechanisms to dominate through- $\sigma$ -bond mechanisms.

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