Deformability of Heme Protein CO Adducts: FT-IR Assignment of the FeCO Bending Mode

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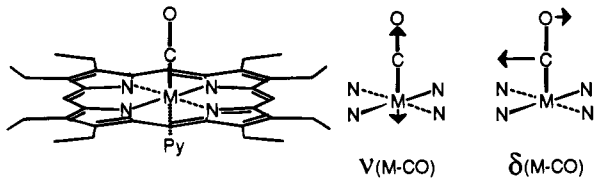
Received August 11, 1994

We report the first assignment of the Fe–C–O bending vibration, at 574 cm^{-1}, in an unconstrained heme model, FeOEP(Py)(CO) (Py, pyridine; OEP, octaethylporphyrin). This is essentially the same frequency as is observed for heme protein CO adducts,[1] implying that the Fe–C–O unit remains linear in the latter, despite distal steric contacts. The IR spectrum rules out recent proposals[2] that the Fe–C–O bending fundamental is actually at a much lower frequency, ~290 or ~360 cm^{-1}. This assignment is important because it reflects the energy required to bend the FeCO linkage.

Assignment of the Fe–C–O bending mode stems from the observation by Yu and co-workers[3] of a 578 cm^{-1} band in the resonance Raman (RR) spectrum of MbCO (Mb, myoglobin), which shows a larger ^{13}CO frequency shift than does the Fe–CO stretching mode, seen at 512 cm^{-1}. The bending frequency is especially sensitive to ^{13}C, because the C atom is strongly displaced in the bending mode (Chart 1). A higher frequency

Chart 1



	ν(M-CO)	δ(M-CO)
M=Fe FeOEP(Py)(CO)	495 cm^{-1}	574 cm^{-1}
M=Ru RuOEP(Py)(CO)	523 cm^{-1}	595 cm^{-1}

for bending than for stretching is unusual among chemical bonds and implies an unusually high bending force constant. This effect is a consequence of the strong backbonding, which dominates the FeCO energetics. Electron donation from the filled Fe(II) $d_{xz,yz}$ orbitals to the CO π^* orbitals is optimum for a linear Fe–C–O unit.[4] Bending of the Fe–C–O angle is therefore strongly resisted. A value of 0.8 mdyn·Å has been estimated[5] for the Fe–C–O bending force constant, from which harmonic energies of 7 or 28 kcal/mol can be calculated for the energy required to impose FeCO angles of 160 or 140°. Energies in this range are unlikely to be generated by protein steric forces, in view of the conformational degrees of freedom of the side chains and the main chain.[6]

These energy estimates are thrown into question, however, by recent proposals that the 578 cm^{-1} RR band is not the bending mode fundamental but rather its overtone[2a] or else a combination band involving the bending mode and a porphyrin vibration.[2b] The rationale for these proposals is that the bending

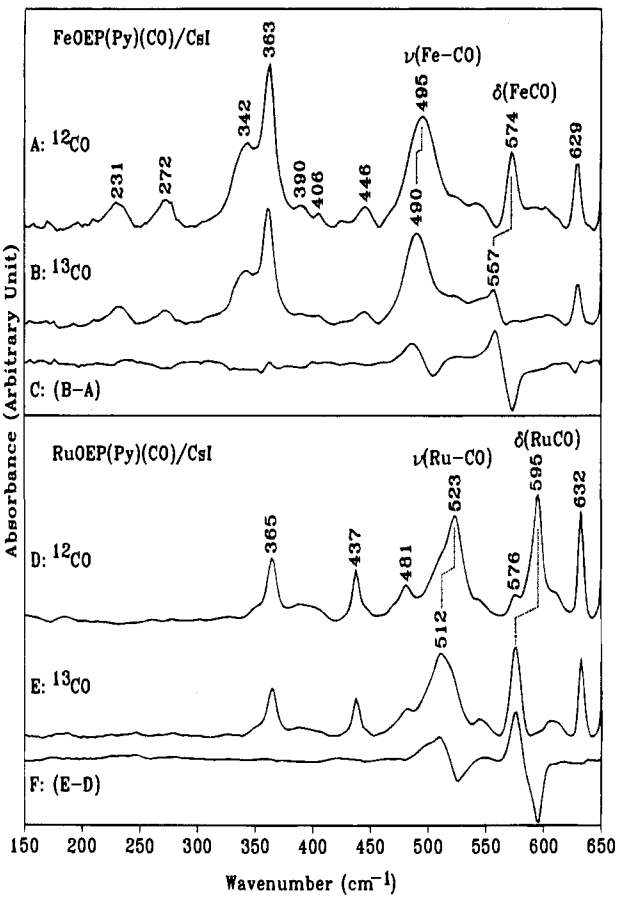


Figure 1. Fourier-transform infrared spectra of FeOEP(Py)(CO), FeOEP(Py)(^{13}CO), RuOEP(Py)(CO), and RuOEP(Py)(^{13}CO) in CsI pellets. The isotope difference spectra show only two isotope-sensitive bands, assigned as indicated. FeOEP(Py)(CO) was synthesized according to a modification of the published procedure.[14] FeOEP(Cl), obtained from Midcentury, was dissolved in THF and reduced by zinc amalgam. Pyridine and CO were added to the filtrated solution. Addition of degassed water precipitated FeOEP(Py)(CO), which was collected by centrifugation and dried over nitrogen. RuOEP(CO)(Py) was prepared by dissolving RuOEP(CO)(THF) (Aldrich) in neat pyridine, followed by precipitation with water. The dried solid was then chromatographed on an alumina column with chloroform as eluent. RuOEP(Py)(^{13}CO) was obtained by photolyzing RuOEP(Py)(CO) in pyridine in the presence of ^{13}CO. The infrared spectra were accumulated for 300 scans with a Nicolet 800 spectrometer.

mode is of *E* symmetry in the idealized C_{4v} point group of CO-heme and lacks a mechanism for RR enhancement.[5,6] A totally symmetric component is present, however, for the overtone and for some combination bands, allowing for RR enhancement. Kitagawa and co-workers[2b] assigned the bending mode fundamental to a very weak band at 365 cm^{-1} in RR spectra of CO adducts of hemoglobin and other heme proteins (but not Mb), which shows the requisite isotope pattern. Tsuboi's overtone proposal[2a] requires that the fundamental be at ~290 cm^{-1}, where it has not been detected. If either of these proposals is correct, then the force constant is much lower than has been estimated, and the energy required to distort the FeCO unit would have to be revised downward.

To resolve this question, we have examined the IR spectrum (Figure 1) of FeOEP(Py)(CO), a heme model with an upright FeCO (Chart 1), as well as its Ru analog. The M–C–O bending and M–CO stretching modes are readily assigned to the bands at 574 and 495 cm^{-1} for M = Fe and at 595 and 523 cm^{-1} for M = Ru, via their ^{13}CO isotope shifts, ~18 cm^{-1} for the bend and ~5 cm^{-1} for the stretch. (The apparently greater isotope shift of the Ru–CO stretch, 11 cm^{-1}, is attributable to

(1) Kerr, E.; Yu, N.-T. In *Biological Applications of Raman Spectroscopy, Vol 3: Resonance Raman Spectra of Heme and Metalloproteins*; Spiro, T. G., Ed.; Wiley: New York, 1988; pp 39–95.
(2) (a) Tsuboi, M. *Ind. J. Pure Appl. Phys.* **1988**, *26*, 188–191. (b) Hirota, S.; Ogura, T.; Shinawa-Itoh, K.; Yoshikawa, S.; Nagai, M.; Kitagawa, T. *J. Phys. Chem.* **1994**, *98*, 6652–6660.
(3) Tsubaki, M.; Srivastava, R. B.; Yu, N.-T. *Biochemistry* **1982**, *21*, 1132.
(4) Hoffman, R.; Chen, M. M. L.; Thorn, D. L. *Inorg. Chem.* **1977**, *16*, 503.
(5) Li, X.-Y.; Spiro, T. G. *J. Am. Chem. Soc.* **1988**, *110*, 6024–6033.
(6) Ray, G. B.; Li, X.-Y.; Ibers, J. A.; Sessler, J. L.; Spiro, T. G. *J. Am. Chem. Soc.* **1994**, *116*, 162–176.

0002-7863/94/1516-11187$04.50/0

an interaction with a porphyrin mode at ~512 cm^{-1}). The Fe−CO stretch has previously been seen at the same frequency in the RR spectrum of the *N*-methylimidazole adduct.[7] The Fe−C−O bending mode has not previously been assigned for unconstrained heme-CO adducts, because the band is missing in their RR spectra. It shows up at ~575 cm^{-1}, however, in RR spectra of some FeCO porphyrins with superstructures that provide distal interactions.[6] Both M−CO stretching and M−C−O bending frequencies are significantly higher for Ru than Fe, reflecting stronger M → CO backbonding by Ru.[8]

No ^{13}CO-sensitive IR band is seen near 360 or 290 cm^{-1} (Figure 1), the alternative positions proposed for the bending mode fundamental.[2] This transition is allowed by IR selection rules, and there is no possibility that the fundamental would be much weaker than a combination or overtone band in the IR spectrum. Consequently, the proposed reassignments are ruled out. The 575 cm^{-1} bending mode fundamental is fully active in the IR, but its activation in RR spectra requires symmetry lowering, which can be provided by off-axis electric fields associated with the binding pocket of heme proteins or the superstructures of constrained porphyrins.[6]

Interest in the Fe−C−O geometry was stimulated by early crystallographic determinations[9] of the MbCO structure, from which the Fe−C−O unit, although disordered, was reported to be distinctly bent, 120−140°. But a nearly linear angle, 169°, was found in a recent X-ray structure of an alternative crystal form of MbCO, in which the bound CO was not disordered.[10] Moreover, a very recent IR polarization measurement on a MbCO single crystal[11] has indicated *no* significant deviation (<10°) from perpendicularity for any of the substates. Off-axis binding of CO has been indicated by IR photoselection experiments, in which the polarization of the C−O stretching band is measured after partial photolysis with a polarized laser beam,[12] but the calculation of the C−O bond vector requires assumptions about the electronic polarizations which may not be valid.

The fact that essentially the same Fe−C−O bending frequency, ~575 cm^{-1}, is seen in MbCO and other proteins and in FeOEP(Py)(CO) strongly implies that the FeCO unit is linear in the proteins. This inference is consistent with analyses of RR vibrational frequencies[5,6] and of NMR chemical shifts,[13] which indicate that frequency variations among heme proteins are explained by changes in Fe → CO backbonding, as modulated by dipolar fields from protein residues, without any indication of geometric distortions. In addition, the RR spectra reveal relatively small frequency differences, 50−80 cm^{-1}, between the Fe−CO stretching and Fe−C−O bending modes, whereas a much larger separation would be expected for bent Fe−C−O because of a strong kinematic interaction between the Fe−C stretching and Fe−C−O bending coordinates.[5]

The preponderance of the evidence now disfavors any significant Fe−C−O bending in heme proteins. The energy required to produce such bending is prohibitive, due to the strong backbonding. On the other hand, the extent of backbonding is sensitive to external influences, making the CO adduct a good spectroscopic probe of proximal and distal effects in the heme pocket.[6]

Acknowledgment. This work was supported by NIH Grant GM 33576.

(7) Kerr, E. A.; Mackin, H. C.; Yu, N.-T. *Biochemistry* **1983**, *22*, 4377−4379.

(8) (a) Stretch and bend assignments for RuOEP(Py)(CO) were previously suggested for bands at 513 and 578 cm^{-1} in its RR spectrum,[8b] but no isotope data were available. These bands are seen as shoulders in the IR spectrum (Figure 1) and are reassigned to porphyrin modes. (b) Kim, D.; Su, Y. O.; Spiro, T. G. *Inorg. Chem.* **1986**, *25*, 3993−3997.

(9) (a) Kuriyan, J.; Wilz, S.; Karplus, M.; Petsko, G. A. *J. Mol. Biol.* **1986**, *192*, 133−154. (b) Cheng, X.; Shoenborn, B. P. *J. Mol. Biol.* **1991**, *220*, 381−399.

(10) Quillin, M. L.; Arduini, R. M.; Olson, J. S.; Phillips, G. N., Jr. *J. Mol. Biol.* **1993**, *234*, 140−155.

(11) Ivanov, D.; Sage, J. T.; Keim, M.; Powell, J. R.; Asher, S. A.; Champion, P. M. *J. Am. Chem. Soc.* **1994**, *116*, 4139−4140.

(12) (a) Moore, J. N.; Hansen, P. A.; Hochstrasser, R. M. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 5062−5066. (b) Ormos, P.; Braunstein, D.; Frauenfelder, H.; Hong, M. K.; Lin, S.-H.; Sauke, T. B.; Young, R. D. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 8492−8496.

(13) Park, K. D.; Guo, K.; Adebodun, F.; Chiu, M. L.; Sligar, S. G.; Oldfield, B. *Biochemistry* **1991**, *30*, 2333−2347.

(14) Peng, S.-M.; Ibers, J. A. *J. Am. Chem. Soc.* **1976**, *98*, 8032−8036.