Nelson, D. J., Theoharides, A. J., Nieburgs, A. C., Murray, R. K., Gonzalez-Fernandez, F., & Brenner, D. (1979) *Int. J. Quantum Chem. 16*, 159.

Pechere, J.-F., Demaille, J., & Capony, J.-P. (1971) *Biochim. Biophys. Acta 236*, 391.

Reuben, J. (1973) *J. Magn. Reson. 11*, 103.

Rhee, M. J., Sudnick, D. R., Arkle, V. K., & Horrocks, W. DeW., Jr. (1981) *Biochemistry 20*, 3328.

Rowan, R., III, McGammon, J. A., & Sykes, B. D. (1974) *J. Am. Chem. Soc. 96*, 4773.

Sowadski, J., Cornick, G., & Kretsinger, R. H. (1978) *J. Mol. Biol. 124*, 123.

Stevens, F. C., Walsh, M., Ho, H. C., Teo, T. S., & Wang, J. H. (1976) *J. Biol. Chem. 251*, 4495–4500.

Vanaman, T. C., Sharief, F., & Watterson, D. M. (1977) in *Calcium Binding Proteins and Calcium Function* (Wasserman, R. H., Corradino, R. A., Carafoli, E., Kretsinger, R. H., MacLennan, D. H., & Siegel, F. L., Eds.) p 107, Elsevier, New York.

Vogel, H. J., Drakenberg, T., & Forsên, S. (1983) in *NMR of Newly Accessible Nuclei* (Laslo, P., Ed.) Academic Press, New York.

Weeds, A. G., & McLachlan, A. D. (1974) *Nature* (*London*) *252*, 646.

Resonance Raman Studies of Carbon Monoxide Binding to Iron "Picket Fence" Porphyrin with Unhindered and Hindered Axial Bases. An Inverse Relationship between Binding Affinity and the Strength of Iron–Carbon Bond[†]

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ABSTRACT: The stretching frequency of the iron–carbon bond, ν(Fe–CO), is a direct measure of the iron–carbon bond strength when there is no change in the Fe–C–O geometry. Here we report resonance Raman detection of ν(Fe–CO) frequencies in the CO complexes of iron(II) α,α,α,α-*meso*-tetrakis(*o*-pivalamidophenyl)porphyrin, Fe^{II}(TpivPP), with trans ligands of varying strength: *N*-methylimidazole (*N*-MeIm), 1,2-dimethylimidazole (1,2-Me_2Im), pyridine (py), and tetrahydrofuran (THF). It was found that the weaker the iron–trans ligand bond, the stronger the iron–carbon bond. Comparisons of sterically hindered (1,2-Me_2Im) and unhindered (*N*-MeIm) bases are of particular interest because of their implication in the phenomenon of hemoglobin cooperativity and the mechanisms of protein control of heme reactivity. While the CO binding affinity of Fe^{II}(TpivPP)(1,2-MeIm) is ~400 times lower than that of Fe^{II}(TpivPP)(*N*-MeIm), the ν(Fe–CO) frequency for the former (at 496 cm^{-1}) is higher than that for the latter (at 489 cm^{-1}). This example shows that the CO binding affinity cannot be directly correlated with the strength of the iron–carbon bond. Comparison of the CO binding to Fe^{II}(TpivPP)(THF) and Fe^{II}(TpivPP)(*N*-MeIm) reveals a similar relationship; the ν(Fe–CO) frequency (at 527 cm^{-1}) in Fe^{II}(TpivPP)(THF)(CO) is 38 cm^{-1} higher than that in Fe^{II}(TpivPP)(*N*-MeIm)(CO), but the CO binding affinity is lower for the THF complex. This implies that the free energy change associated with CO binding is not localized at the Fe–C bond; there must be a charge redistribution upon ligand binding so that some free energy compensation has occurred among many chemical bonds.

Carbon monoxide is an excellent Raman–visible ligand for probing the nature of active sites in hemoproteins (Tsubaki et al., 1982). It binds to practically any ferro heme reversibly, and its geometry is subject to distal steric effect (Huber et al., 1970; Norvell et al., 1975; Padlan & Love, 1975; Heidner et al., 1976; Steigemann & Weber, 1979; Baldwin, 1980). Tsubaki et al. (1982) first identified the Fe–CO stretching, Fe–C–O bending, and C–O stretching vibrations in the resonance Raman spectra of carbonmonoxy human hemoglobin A and carbonmonoxy sperm whale myoglobin. The Fe–CO stretching vibration, readily detectable upon Soret excitation, is of particular interest and importance because of its anticipated sensitivity to geometry and to both σ and π back-bonding. The bound C–O stretching vibration has been extensively studied by infrared spectroscopy (Alben & Caughey, 1968; Caughey et al., 1969; Caughey, 1970; Caughey et al., 1973; Barlow et al., 1976; Yoshikawa et al., 1977; Makinen et al., 1979; O'Keefe et al., 1978; Caughey et al., 1978; Caughey, 1980; Alben et al., 1981). However, it may respond more to changes in the π back-bonding than to the electron density in the Fe–CO σ bond.

In recent years considerable research has been directed toward elucidating the detailed structure of the heme, its immediate environment in various hemoproteins, and ligand binding affinities. Much effort has now been focused on the synthesis and study of heme models capable of coordinating molecular oxygen reversibly without autoxidation of Fe^{II} to Fe^{III}. The so-called "picket fence" porphyrins (Collman et al., 1975) with hydrophobic pickets are capable of binding dioxygen and carbon monoxide reversibly at room temperature and thus may be considered as an excellent model system for hemoglobin and myoglobin.

Resonance Raman studies of dioxygen binding to picket fence porphyrins have been reported (Burke et al., 1978; Walters et al., 1980; Hori & Kitagawa, 1980). In this paper, we study the binding of CO to picket fence porphyrins to examine the influence of a trans ligand upon the strength of the Fe–C bond. The effect of distal steric hindrance on the Fe–C bond in carbonmonoxy "strapped hemes" has been reported elsewhere (Yu et al., 1983).

In the absence of steric hindrance as in the case of picket fence porphyrins, the Fe–C–O linkage is presumably linear

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and perpendicular to the heme plane (Peng & Ibers, 1976; Scheidt et al., 1981). Without a geometry change, the ν(Fe–CO) stretching frequency is a good indicator of the Fe–C bond strength. This is true among complexes with similar potential well shapes where a linear relation exists between bond strength and force constant (for Morse potential function). Therefore, a stronger bond has a greater force constant which gives rise to a higher stretching frequency. Comparison of CO complexes with hindered (1,2-Me_2Im)[1] and unhindered (N-MeIm) bases reveals that the tension of the iron–imidazole bond increases the Fe–C bond strength. In other words, the weaker the trans Fe–ligand bond, the stronger the Fe–C bond. This relation holds for a weak ligand such as tetrahydrofuran; there the Fe–C bond was found to be unusually strong (hence short). We also have detected a very strong Fe–C bond in pentacoordinated carbonmonoxy picket fence porphyrin without a trans ligand. Studies on CO binding to iron(II) octaethylporphyrin and iron(II) tetraphenylporphyrin with hindered and unhindered bases are also reported. We demonstrate that a lower CO binding affinity can mean a stronger Fe–C bond. The difference in the direction of ν(Fe–ligand) frequency shifts between CO and O_2 binding to picket fence porphyrin with hindered and unhindered bases is discussed in terms of σ and π bonding between iron and axial ligands.

Materials and Methods

The "picket fence" porphyrin iron(III) $\alpha,\alpha,\alpha,\alpha$-*meso*-tetrakis($o$-pivalamidophenyl)porphyrin bromide [Fe^{III}(TpivPP)Br] was synthesized by Midcentury Inc., Posen, IL, according to the method of Collman et al. (1975). The sample was chromatographically pure, with little isomerization. The CO complexes of these hemes (50 μM) were prepared with N-methylimidazole (250 μM), 1,2-dimethylimidazole (600 μM), and pyridine (750 μM) as axial bases in benzene solutions at room temperature. The complex with tetrahydrofuran (THF) was prepared by the dissolution of the porphyrin (50 μM) in neat THF while the 5-coordinate complex, without a trans ligand, was prepared by dissolution in neat benzene. The sample solution (1 mL) was placed into a cylindrical quartz Raman cell. The reduction was carried out by the addition of a minimum amount of a reducing solution prepared by the 10-fold dilution with benzene of Vitride T reducing agent (J. T. Baker Chemical Co., Phillipsburg, NJ; 70% $C_6H_{16}AlNaO_4$ in toluene). The cell was immediately sealed with a rubber septum and evacuated. Carbon monoxide or oxygen was then introduced at $\sim$1 atm. The isotope-substituted complexes were prepared similarly with $^{13}C^{16}O$ (Bio-Rad Laboratories, Richmond, CA; 93.1 atom % ^{13}C), $^{12}C^{18}O$ (Stohler Isotope Chemicals, Waltham, MA; 99 atom % ^{18}O), and $^{13}C^{18}O$ (Prochem US Services Inc., Summit, NJ; 91.7 atom % ^{13}C and 98.5 atom % ^{13}C) each at an initial pressure of one atmosphere.

Iron(III) *meso*-tetraphenylporphyrin bromide, Fe^{III}(TPP)Br, was purchased from Midcentury Inc., Posen, IL, and iron(III) octaethylporphyrin chloride, Fe^{III}(OEP)Cl, was obtained from Strem Chemicals, Inc., Newburyport, MA. The CO complexes of these hemes ($\sim$110 μM) were prepared in a manner similar to that described above with 550 μM N-methylimidazole (N-MeIm) and 1.7 × 10^4 μM 1,2-dimethylimidazole (1,2-Me_2Im) in benzene.

All reagents were purified immediately prior to use and stored under prepurified N_2. Benzene was stirred over concentrated sulfuric acid to remove organic impurities, neutralized with a dilute $NaHCO_3$ solution, dried over anhydrous $MgSO_4$, and distilled from sodium benzophenone ketyl. Pyridine and N-methylimidazole (Sigma Chemical Co., St. Louis, MO) were distilled from KOH; 1,2-dimethylimidazole (Aldrich Chemical Co., Milwaukee, WI) was distilled from Na; THF was distilled from sodium benzophenone ketyl. Preparations requiring an inert atmosphere were performed in a controllable atmosphere globe box under N_2.

All Raman spectra were obtained by using a highly sensitive multichannel laser Raman system, which consists of a modified Spex 1402 0.85 m Czerny–Turner double monochromator (600 grooves/mm in additive dispersion), a Princeton Applied Research (Princeton, NJ) Model 1254 dry ice cooled SIT (silicon intensified target) multichannel detector , a Princeton Applied Research Model 1216 detector controller, a Tektronix (Beaverton, OR) Model 604 monitor, and a Princeton Applied Research Model 1215 OMA 2 microprocessor-based console (Yu & Srivastava, 1980). The 406.7-nm line of a Spectra-Physics (Mountain View, CA) Model 171 krypton ion laser was employed for excitation with laser power approximately 10 mW at the sample. The sample in the rotating Raman cell was spun throughout the measurements to prevent local heating and to lessen photodissociation. Scattered light was focused onto the entrance slit of the spectrometer which was opened 100 μ in width and 0.2 cm in height. Fenchone was used to calibrate all spectra, and wavenumbers reported are accurate to ±1 cm^{-1} for sharp lines and ±2 cm^{-1} for broad lines.

Results and Discussion

Identification of ν(Fe–CO) Stretching Vibration: Comparison between Picket Fence Heme and Hemoproteins. In Figure 1, we show the effects of carbon monoxide isotope substitution on the low-frequency region (100–700 cm^{-1}) of Fe^{II}(TpivPP)(N-MeIm)(CO) spectra. The substitutions clearly reveal an isotope sensitive line at 489 cm^{-1} for Fe^{II}(TpivPP)(N-MeIm)($^{12}C^{16}O$) which shifts to 485 cm^{-1} upon substitution by $^{13}C^{16}O$, to 481 cm^{-1} by $^{12}C^{18}O$, and to 447 cm^{-1} by $^{13}C^{18}O$. No additional lines appear sensitive to carbon monoxide isotope substitution experiments. The ν(Fe–CO) frequency of 489 cm^{-1} is surprisingly low compared to the values of 507 and 512 cm^{-1} found by Tsubaki et al. (1982) for the ν(Fe–CO)'s of (carbonmonoxy)-HbA and (carbonmonoxy)-Mb, respectively. Indeed, with a strong field ligand such as N-MeIm, a ν(Fe–CO) stretching in benzene appears 23 cm^{-1} (18 cm^{-1}) lower than that of MbCO (HbCO) despite the much higher CO affinity for the model heme (Collman et al., 1976). In fact, Collman et al. (1976, 1979) have reported the CO binding to Fe^{II}(TpivPP)(N-MeIm)(CO) to be extremely strong, with $P_{1/2} \approx 10^{-5}$ torr in toluene and "irreversible" in the solid state. This frequency difference is, at least in part, due to the geometry change from a linear Fe–CO group perpendicular to the porphyrin plane in picket fence complexes to a bent and/or tilted bonding configuration of Fe–C–O found in hemoproteins. Apparently, bulky amino acid residues near the binding site (in hemoproteins) distort the Fe–CO geometry and lower the affinity ratio of CO vs. O_2, relative to the unconstrained heme models. This is thought to be responsible for the partial detoxification of CO in respiratory biological systems (Caughey, 1970; Perutz, 1976; Collman et al., 1976, 1979).

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[1] Abbreviations: Fe^{II}(deutero), iron(II) deuteroporphyrin IX dimethyl ester; Fe^{II}(TPP), iron(II) *meso*-tetraphenylporphyrin; Fe^{II}(TpivPP), iron(II) $\alpha,\alpha,\alpha,\alpha$-*meso*-tetrakis($o$-pivalamidophenyl)porphyrin; Fe^{II}-[Piv_3(5CImP)Por], iron(II) $5\alpha,10\alpha,15\alpha$-*meso*-tri($o$-pivalamidophenyl)-$20\beta$-[$o$-[5-(1-imidazolyl)valeramido]phenyl]porphyrin; Fe^{II}(OEP), iron(II) octaethylporphyrin; N-MeIm, N-methylimidazole; 1,2-Me_2Im, 1,2-dimethylimidazole; py, pyridine; THF, tetrahydrofuran; $P_{1/2}$, pressure at half-saturation.

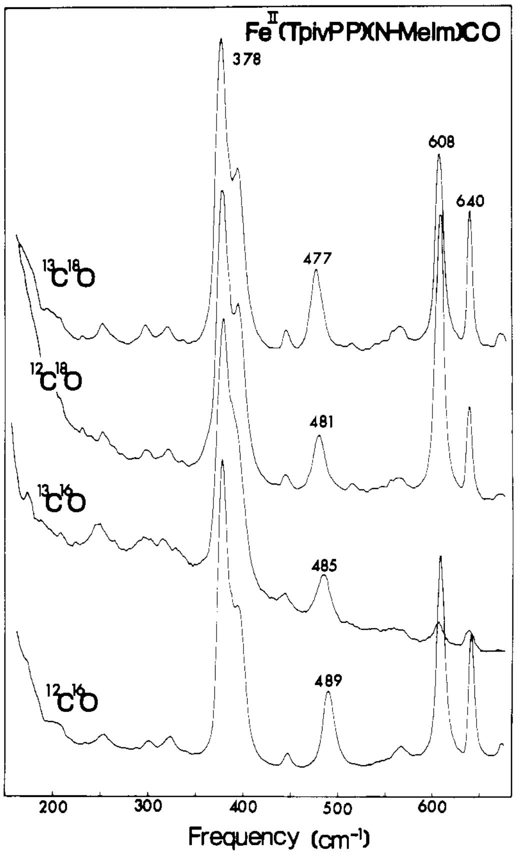

FIGURE 1: Carbon monoxide isotope effects on the low-frequency region (100–700-cm^{-1}) spectra of Fe^{II}(TpivPP)(*N*-MeIm)(CO). λ_{exc} = 406.7 nm; concentration 50 μM. Benzene line at 608 cm^{-1}.

Recent studies by Yu et al. (1983) on strapped hemes with steric hindrance (provided by a hydrocarbon chain strapped across one face of the heme) also show the higher ν(Fe–CO) frequencies. When the "strap" is shortened, a greater sideway shearing strain is applied to the CO ligand, resulting in lower binding affinity, but higher ν(Fe–CO) frequencies for the greater CO distortion.

Lack of δ*(Fe–C–O) Enhancement in Sterically Unhindered Hemes.* Attempts were made to identify the δ(Fe–C–O) bending vibration in Fe^{II}(TpivPP)(*N*-MeIm)(CO). Careful examination of the region around ν(Fe–CO) reveals no δ(Fe–C–O), although early in this study the peak at ~566 cm^{-1} was suspected. However, this line appears insensitive to isotope substitution and does not show the expected decrease–increase–decrease "zigzag" frequency shift pattern detected in both MbCO (577 cm^{-1}) and HbCO (578 cm^{-1}) (Tsubaki et al., 1982). We report, therefore, no enhancement of the δ(Fe–C–O) bending vibration in this picket fence system. Recent work with sterically hindered carbonmonoxy "strapped" hemes revealed resonance Raman enhancement of the Fe–C–O bending mode upon Soret excitation. The results clearly show that the distortion of the Fe–C–O linkage enhances the intensity of the Fe–C–O bending mode relative to that of the Fe–CO stretching mode. When the strap is removed and the distortion is relieved, the bending mode is no longer detected. Thus, if we assume that the picket fence system binds CO to Fe in a linear and perpendicular fashion due to the absence of any steric hindrance, then the absence of the δ(Fe–C–O) line is consistent with the results for the simple "unstrapped" iron porphyrin (heme 5) (Yu et al., 1983).

Solvent Effects on ν*(Fe–CO) Vibration.* The iron(II) porphyrins, Fe^{II}(TPP)(py)(CO) and Fe^{II}(TpivPP)(py)(CO) are both meso-substituted tetraarylporphyrins. The picket fence porphyrin, however, was developed with a protective enclosure on one side of the porphyrin in order to favor 5-coordination, i.e., leaving a hydrophobic pocket for ligand complexation and to discourage rapid bimolecular irreversible autoxidation of Fe^{II} to Fe^{III} in ferrous dioxygen complex preparation. Our study exhibits the independence of the ν(Fe–CO) for picket fence CO complexes to solvent. In order to prove this point, spectra of Fe(TPP)(py)(CO) were obtained in C_6H_6 and CH_2Cl_2 and compared to similar Fe(TpivPP)(py)(CO) spectra. For Fe(TPP)(py)(CO) (an unprotected model system), we observed a ν(Fe–CO) at 484 cm^{-1} in C_6H_6 which shifts 6 cm^{-1} higher to 490 cm^{-1} in CH_2Cl_2 (spectra not shown). However, for Fe(TpivPP)(py)(CO), a ν(Fe–CO) is seen at 486 cm^{-1} in C_6H_6 (see Figure 2) and at 487 cm^{-1} in CH_2Cl_2. Thus, no noticeable change in ν(Fe–CO) for the picket fence complex is observed (within ±1 cm^{-1}). Due to the insensitivity of the Fe–CO stretching frequency to solvent, we conclude that the picket fence cavity protects carbon monoxide from significant interactions with the solvent. The ligand is buried in the protected binding pocket in these complexes and therefore solvation effects are largely eliminated. In simple "flat porphyrins" such as Fe^{II}(TPP)(py)(CO), however, the ligand is exposed to the solvent and shows a marked dependence on the physical properties of the solvent. In fact, infrared results show that for unprotected heme–CO complexes the ν(C–O) frequency decreases by ~15–20 cm^{-1} as the dipole moment of the solvent increases from 0 to 4 D (Barlow et al., 1976).

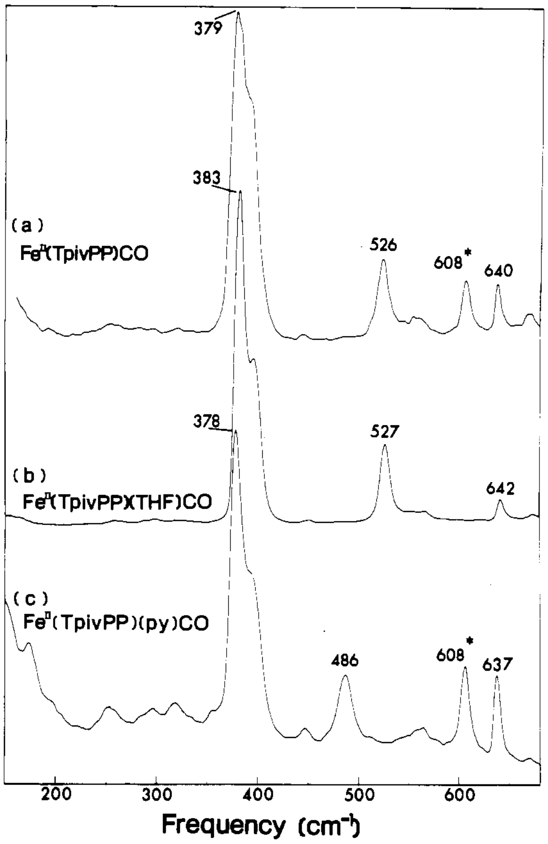

FIGURE 2: Effects of ligand field strength of the trans ligand on ν(Fe–CO) in the resonance Raman spectra of carbonmonoxy iron(II) "picket fence" complexes in the 100–700-cm^{-1} region: (a) no trans ligand, benzene solution; (b) neat THF; (c) pyridine in benzene solution. λ_{exc} = 406.7 nm; concentration 50 μM.

An Unusually Strong Fe–C Bond When the Trans Fe–Ligand Bond Is Weak or Absent. Due to the absence of CO distortion, the ν(Fe–CO) frequency is indicative of the iron–

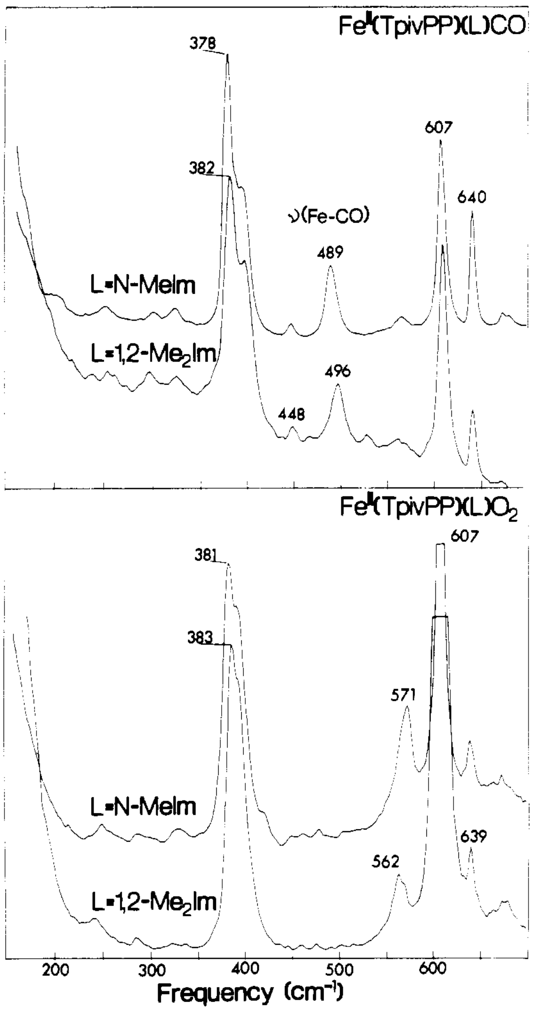

FIGURE 3: Comparison of the effects of proximal base tension on ν(Fe–CO) (upper panel) and ν(Fe–O$_2$) (lower panel) of iron(II) picket fence porphyrins in the 100–700-cm^{-1} region: (a) Fe(TpivPP)(*N*-MeIm)(CO); (b) Fe(TpivPP)(1,2-Me$_2$Im)(CO); (c) Fe(TpivPP)(*N*-MeIm)(O$_2$); (d) Fe(TpivPP)(1,2-Me$_2$Im)(O$_2$). λ_{exc} = 406.7 nm; concentration 50 μM. Benzene line at 607 cm^{-1}.

Table I: Stretching Frequencies (cm^{-1}) for CO and O$_2$ Complexes of Fe^{II}(TpivPP), Fe^{II}(OEP), and Fe^{II}(TPP) Porphyrins

Fe^{II}(TpivPP)(*N*-MeIm)(CO)

isotope	ν(Fe–CO)	isotope	ν(Fe–CO)
$^{12}C^{16}O$	489	$^{12}C^{18}O$	481
$^{13}C^{16}O$	485	$^{13}C^{18}O$	477

Effect of Trans Ligand Field Strength Fe^{II}(TpivPP)(R)(CO)

R	ν(Fe–CO)	ν(Fe–^{13}CO)	ν(Fe–C^{18}O)	ν(Fe–^{13}C^{18}O)
none	526			512
THF	526	521	516	511
pyridine	486	482	477	474
N-MeIm	489	485	481	477
1,2-Me$_2$Im	496	491		

Effects of Proximal Base Tension

	ν(Fe–O$_2$)	ν(Fe–CO)
Fe(TpivPP)(*N*-MeIm)(O$_2$)	571	
Fe(TpivPP)(1,2-Me$_2$Im)(O$_2$)	562	
Fe(TpivPP)(*N*-MeIm)(CO)		489
Fe(TpivPP)(1,2-Me$_2$Im)(CO)		496
Fe(OEP)(*N*-MeIm)(CO)		496
Fe(OEP)(1,2-Me$_2$Im)(CO)		501
Fe(TPP)(*N*-MeIm)(CO)		486
Fe(TPP)(1,2-Me$_2$Im)(CO)		494

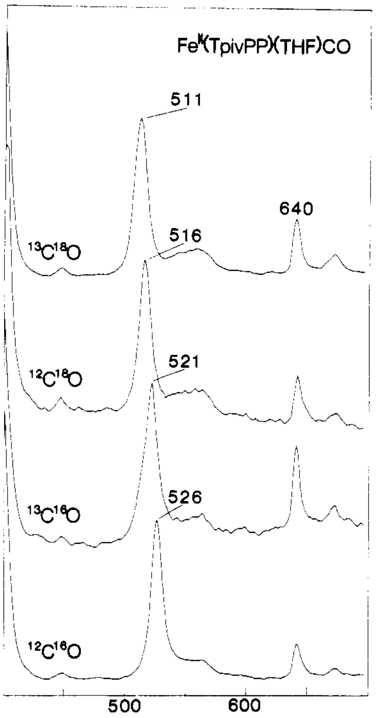

FIGURE 4: Carbon monoxide isotope effects on the low-frequency region (400–700-cm^{-1}) spectra of Fe^{II}(TpivPP)(THF)(CO). λ_{exc} = 406.7 nm; concentration 50 μM. Neat THF solution.

carbon bond strength. This notion is derived from diatomic molecules in which the force constant for stretching a bond is correlated with its dissociation energy (hence, bond strength) and length (equilibrium internuclear distance) (Lesk, 1982). A stronger bond is likely to be shorter and vibrate with a higher frequency. Here we investigate factors affecting the strength of the Fe–C bond by reporting the change in the ν(Fe–CO) frequency brought about by altering the properties of the fifth (proximal) heme ligand. Both strong and weak trans ligands have been compared, including *N*-MeIm, 1,2-Me$_2$Im, pyridine, and THF. We have also detected the ν(Fe–CO) frequency from 5-coordinate Fe^{II}(TpivPP)(CO) without a trans ligand. Important results are shown in Figures 2 and 3 and summarized in Table I.

Our studies reveal unambiguously the trans (proximal) base influence on the strength of the Fe–C bond. It is plausible that the frequency shifts could derive from a change in the composition of the normal mode (predominantly but not purely an Fe–C vibration) as the trans ligand is changed. However, isotope substitution studies for two complexes Fe^{II}(TpivPP)(*N*-MeIm)(CO) and Fe^{II}(TpivPP)(THF)(CO) which have very different trans ligands show practically identical isotope shifts. If the mode composition did change, the isotope shifts would change. For Fe^{II}(TpivPP)(THF)(CO), the ν(Fe–CO) frequency appears at 526 cm^{-1} for $^{12}C^{16}O$ and shifts to 521 cm^{-1} upon substitution by $^{13}C^{16}O$, to 516 cm^{-1} by $^{12}C^{18}O$, and to 511 cm^{-1} by $^{13}C^{18}O$ (Figure 4). Within our ±1 cm^{-1} experimental accuracy, these isotope shifts are nearly the same as those observed for Fe^{II}(TpivPP)(*N*-MeIm)(CO). This implies that the composition of the normal mode is not appreciably altered by the different trans ligand. The frequency change observed here by changing the trans ligand then is ascribed to a weakening or strengthening of the Fe–C bond and does not involve any geometry change (distortion) of the bound carbon monoxide or significant change of the normal mode composition.

In binding to iron, carbon monoxide acts as a σ donor and as a π acceptor (Rougee & Brault, 1975). Heme iron serves as a σ acceptor and a π donor, and its ability to serve in both capacities determines the strength of the Fe–C bond and can result from changes in the porphyrin and/or trans ligand (Yoshikawa et al., 1977). Our studies reveal that the weaker the proximal Fe–ligand bond, the stronger the Fe–CO bond. Tetrahydrofuran represents one of the weakest field and weakest binding ligands and leads to a stronger than usual bonding of CO to heme. The ν(Fe–CO) frequency for the Fe^{II}(TpivPP)(THF)(CO) appears at 527 cm^{-1}. With a strong field ligand such as *N*-MeIm or pyridine, the ν(Fe–CO) appears at $\sim$489 cm^{-1} which is 38 cm^{-1} lower than weak field ligand. X-ray crystallographic data of Scheidt et al. (1981) characterized the Fe^{II}(deutero)(THF)(CO) complex and indeed reported an unusually short Fe–C bond distance of 1.706 Å compared to the 1.77 (3) Å value observed in Fe^{II}(TPP)(py)(CO) by Peng & Ibers (1976). This demonstrates that the ν(Fe–CO) frequency sensitivity to bond length changes is $\sim$6 cm^{-1}/0.01 Å assuming an inverse linear relationship.[2] The sensitivity of bond strength between CO and iron is also evidenced by reported infrared ν(C–O) stretching frequencies. In Fe(deutero)(THF)(CO) the ν(C–O) is 1955 cm^{-1}, considerably lower than that observed for related deuteroporphyrin derivatives, e.g., 1973 cm^{-1} in Fe(deutero)(py)(CO) in $CHBr_3$ (Alben & Caughey, 1968), 1973 cm^{-1} in [Fe(CO)-deutero)]$_2$(μ-hydrazine) in $CHCl_3$ (Caughey et al., 1972), and 1980 cm^{-1} in Fe(TPP)(py)(CO) (Scheidt et al., 1981). In general, the higher the ν(Fe–CO) frequency, the lower the ν(C–O) frequency (Yu et al., 1983). Another important feature to be emphasized here is that in distinct contrast to the carbonmonoxy hemoproteins where CO is tilted (or bent) off axis, the Fe–C–O vectors are within 2° of being perpendicular to the heme plane; the Fe–C–O angles are 178.3° in Fe^{II}(deutero)(THF)(CO) (Scheidt et al., 1981) and 179 (2)° in Fe^{II}(TPP)(py)(CO) (Peng & Ibers, 1976). Thus, in the absence of steric hindrance, it seems safe to assume that the Fe–C–O linkage is essentially linear and perpendicular.

The 5-coordinate complex, Fe^{II}(TpivPP)(CO), free of a trans ligand, further illustrates the significant influence on the Fe–CO bond strength by the nature of the ligand trans to the carbon monoxide. The ν(Fe–CO) frequency appears at 526 cm^{-1} (see Figure 2), indicative of an unusually short Fe–C bond. It shifts to 512 cm^{-1} upon substitution by $^{13}C^{18}O$, a total of 14 cm^{-1}, which is comparable to those observed in the corresponding THF and *N*-MeIm complexes. The results agree with X-ray studies that report an Fe–CO distance of 1.694 (4) Å for a 5-coordinate macrocyclic species (Goedken et al., 1976). Although the bond length is even shorter than that observed for THF complexes, we observe no further increase in the ν(Fe–CO) stretching frequency. The composition of the normal mode giving rise to the Fe–CO vibration is primarily the same in all the carbonmonoxy complexes; however, there may exist some slight difference between the pentacoordinate complex and the hexacoordinate complexes. The mode composition is very similar among the various 6-coordinate species, yet for the 5-coordinate species, it may be somewhat (slightly) different due to the absence of a trans ligand or due to the possibility of the iron being further from the porphyrin plane.

Experimentally, extreme care was employed to ensure a high-quality low-frequency spectrum of the Fe^{II}(TpivPP)(CO) complex. Rigorous precautions were utilized to purify all reagents since preliminary studies revealed that any slight impurities interfered with obtaining a true resonance Raman spectrum of the 5-coordinate species. In the spectrum we detected only a single Fe–CO stretching line auspicious of the 5-coordinate species. The formation of the 6-coordinate Fe^{II}(TpivPP)(CO)$_2$ complex is possible under our experimental conditions (Wayland et al., 1978; Rougee & Brault, 1975). The equilibrium binding constant for the monocarbonyl adduct of Fe^{II}(TPP) is at least 400 times greater than that for the dicarbonyl adduct (Wayland et al., 1978). In studies with very low CO pressure ($P < 100$ torr), the line at 526 cm^{-1} is the only isotope-sensitive line observed. Increasing the pressure to greater than 1 atm, the same single ν(Fe–CO) line is observed with no additional line appearing. The peak is assigned to the ν(Fe–CO) for a 5-coordinate species. Since a complex without a trans ligand has approximately the same Fe–C bond length as a complex with the weak trans THF ligand, a similar frequency is expected. It is likely that with the 406.7-nm excitation, the ν(Fe–CO) stretching vibration from the biscarbonyl adduct is not enhanced. In the Fe^{II}(OEP) system, however, we have observed a second isotope-sensitive line characteristic of an Fe–CO stretching vibration because of its monotonous isotope shift behavior with increasing CO mass (E. A. Kerr and N.-T. Yu, unpublished results).

A Tension on Trans Iron–Imidazole Bond Increases the Fe–C Bond Strength but Decreases the CO Binding Affinity. A central feature of the Hoard–Perutz stereochemical mechanism for the cooperativity of hemoglobin (Hoard & Scheidt, 1973; Perutz, 1970) is the lessened O_2 affinity of the T (low-affinity) state presumed to be caused by the protein-induced restraint on the proximal histidine. To prove the nature of this steric restraint experimentally, studies have turned to sterically hindered axial bases (Rougee & Brault, 1975). In this study, iron picket fence porphyrins with unhindered (*N*-MeIm) and hindered (1,2-Me$_2$Im) imidazoles are employed. The unconstrained *N*-MeIm complex provides a reference for unconstrained (or relaxed) hemoproteins (Rougee & Brault, 1975). In the sense that the 2-methyl group of 1,2-Me$_2$Im provides restraint to the motion of the axial base toward the porphyrin upon oxygenation, Fe^{II}(TpivPP)(1,2-Me$_2$Im) is a model for the T form of Hb. The presence of such a steric restraint with 1,2-Me$_2$Im is evidenced by the existence of 5-coordinate ferrous porphyrins (Hoard & Scheidt, 1973) and of a slightly higher frequency for the Fe^{II}–N$_\epsilon$ (*N*-MeIm) stretching vibration (225 cm^{-1}) than the Fe^{II}–N$_\epsilon$ (2-MeIm) stretching frequency (209 cm^{-1}) (Hori & Kitagawa, 1980). The O_2 affinity of the *N*-MeIm adduct ($P_{1/2} = 0.3$ torr) is similar to that of Mb, or R (high-affinity) state Hb. Furthermore, it is interesting that the hindrance offered by the 2-methyl group lowers the affinity of the 1,2-Me$_2$Im adduct to the level found in the T state Hb ($P_{1/2} = 38$ torr) (Collman et al., 1978a,b).

In the case of oxy complexes when the proximal tension is increased by changing from *N*-MeIm (unhindered) to 1,2-Me$_2$Im (hindered), the ν(Fe–O$_2$) decreases from 571 to 562 cm^{-1} (in benzene) (see Figure 3). This confirms the earlier report by Walters et al. (1980) that there is a 4-cm^{-1} difference in ν(Fe–O$_2$) between Fe^{II}(TpivPP)(*N*-MeIm)(O$_2$) and Fe^{II}(TpivPP)(1,2-Me$_2$Im)(O$_2$) (in CH_2Cl_2) and contrasts the insensitivity of ν(Fe–O$_2$) to such a proximal tension in the same heme system found by Hori & Kitagawa (1980).

The CO affinity of Fe^{II}(TpivPP)(1,2-Me$_2$Im) is known to be appoximately 400 times lower than that of the unconstrained complex, iron(II) 5α,10α,15α-*meso*-tri(*o*-pival-

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[2] On the basis of Badger's rule, the relationship between equilibrium bond length (r_e) and bond stretching frequency ($\tilde{\nu}$) is $r_e) = a + b\,\tilde{\nu}^{-2/3}$, where a and b are constants.

amidophenyl)-20β-[*o*-[5-(1-imidazolyl)valeramido]phenyl]-porphyrin, $Fe^{II}[Piv_3(5CImP)]$, an imidazole-tailed picket fence porphyrin (Collman et al., 1979). We expect no large difference between the CO affinity of $Fe^{II}[Piv_3(5CImP)]$-porphyrin and Fe^{II}(TpivPP)(*N*-MeIm), two very similar sterically unhindered complexes. Quite interestingly, our data indicate that the Fe–C bond is stronger for the lower affinity complex. The overall free energy change ($\Delta G°$) associated with ligand binding in heme systems, which determines the binding affinity, is not necessarily localized at the iron–CO bond. In comparing the *N*-MeIm and 1,2-Me_2Im complexes the free energy difference between unligated Fe^{II}(TpivPP)-(*N*-MeIm) and ligated Fe^{II}(TpivPP)(*N*-MeIm)(CO) must be greater than the free energy difference between Fe^{II}-(TpivPP)(1,2-Me_2Im) and Fe^{II}(TpivPP)(1,2-Me_2Im)(CO) by ~3.5 kcal mol^{-1}. This extra free energy associated with the higher affinity complex can be accounted for by considering the free energy differences between the two unligated species and between the two ligated species: $-\Delta G = [G°_u(N\text{-MeIm}) - G°_l(N\text{-MeIm})] - [G°_u(1,2\text{-Me}_2\text{Im}) - G°_l(1,2\text{-Me}_2\text{Im})] = [G°_l(1,2\text{-Me}_2\text{Im}) - G°_l(N\text{-MeIm})] - [G°_u(1,2\text{-Me}_2\text{Im}) - G°_u(N\text{-MeIm})] > 0$. The difference between ligated Fe^{II}-(TpivPP)(1,2-Me_2Im)(CO) and Fe^{II}(TpivPP)(*N*-MeIm)(CO) must be greater (by ~3.5 kcal mol^{-1}) than the difference between unligated Fe^{II}(TpivPP)(1,2-Me_2Im) and unligated Fe^{II}(TpivPP)(*N*-MeIm). Since the unligated Fe^{II}(TpivPP)-(1,2-Me_2Im) is a weaker complex and presumably has a higher free energy (hence less stable) than the unligated Fe^{II}-(TpivPP)(*N*-MeIm), the ligated Fe^{II}(TpivPP)(1,2-Me_2Im)-(CO) must have a higher free energy than the ligated Fe^{II}-(TpivPP)(*N*-MeIm)(CO). If the formation of the Fe–C bond were an isolated event involving no changes in other parts of the complex, the Fe–C bond in the low-affinity (1,2-Me_2Im) complex must be weaker than that in the high-affinity (*N*-MeIm) complex. Since the reverse relation was observed, the extra chemical bond energy gained in the Fe–C bond in the low-affinity complex must be compensated by a greater energy loss in the rest of the complex. This energy compensation is the result of charge redistribution upon ligand binding through the σ- and π-bonding network of the axial ligands and porphyrin macrocycle. It may be easy to see the partial energy compensation between Fe–C and C–O bonds: the stronger the Fe–C bond, the weaker the C–O bond. Comparison of X-ray crystallographic data from several heme and non-heme iron–CO systems reveals that when the Fe–C bond length increases from 1.694 to 1.706, 1.730, 1.751, and 1.77 Å, the C–O bond distance decreases from 1.157 to 1.144, 1.146, 1.137, and 1.12 Å) (Goedken et al., 1976; Scheidt et al., 1981; Peng & Ibers, 1976). Thus, it is important to realize that the ligand binding affinity in heme systems should *not* be correlated with the strength of the Fe–ligand bond. The Fe–ligand stretching frequency should *not* be used as the sole indicator of binding affinity. In the case of a THF complex such as Fe^{II}(deutero)(THF)(CO), the binding constant for the reaction (in benzene) Fe^{II}(deutero)(THF) + CO → Fe^{II}(deutero)-(THF)(CO) is 2.8×10^6 M^{-1} which is much lower than the corresponding constant of 4.8×10^8 M^{-1} for an imidazole ligand (Rougee & Brault, 1975). The Fe–C bond length for the THF complex is much shorter (Scheidt et al., 1981) and hence stronger. Although we do not have resonance Raman data for the deutero heme system, our present study on the picket fence system shows the same relationship to hold in comparison of *N*-MeIm and THF complexes.

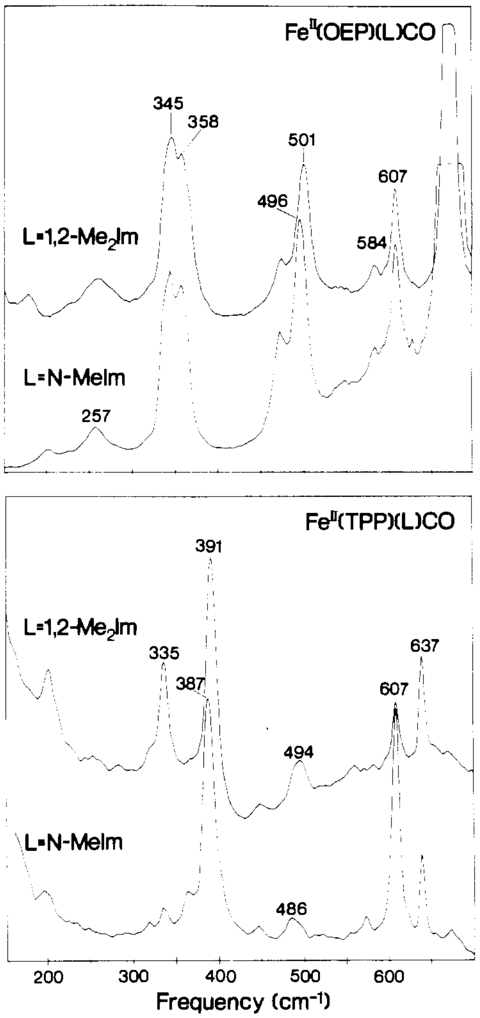

FIGURE 5: Comparison of the effects of proximal base tension on ν(Fe–CO) of carbonmonoxy complexes of iron(II) octaethylporphyrin (upper panel) and iron(II) tetraphenylporphyrin (lower panel). λ_{exc} = 406.7 nm; concentration 110 μM. Benzene line at 607 cm^{-1}.

The data from *N*-MeIm and 1,2-Me_2Im complexes may be rationalized in terms of the relative σ- and π-donating character of the imidazole ligands. In σ bonding, the constrained Fe–N_ϵ (proximal bond) of the 1,2-Me_2Im complex donates less electron density through the σ network than does the *N*-MeIm complex. Therefore, the σ bonding between iron and CO will increase due to the competition from both sides for the same Fe (d_{z^2}) orbitals. This similarly occurs with O_2 binding, thereby strengthening the Fe–O σ bonds. In terms of π donation, the substantially stretched Fe–N_ϵ bond of the 1,2-Me_2Im complexes decreases the imidazole to iron π donation which decreases the strength of the d_π(Fe)–π*(ligand) interactions for both gaseous ligands, O_2 and CO. With O_2 binding, the ν(Fe–O_2) decreases for the constrained complex, thus indicating that a large decrease in π bonding between Fe and O_2 more than counterbalances the increase in σ bonding. In the constrained complex where the ν(Fe–CO) increases, the reverse behavior must occur; i.e., the increase in Fe–C σ bonding more than compensates the decrease in π bonding. As a result of the π*(CO) orbitals being higher in energy than the π*(O_2) orbitals, the interaction between d_π(Fe) and π*-(CO) is weaker than that between d_π(Fe) and π*(O_2). Hence, the differentiation could be the result of O_2 depending more strongly upon the π basicity of the proximal base than does CO (Chang & Traylor, 1973). It is noted that the validity of the assignment of the Fe^{II}–O_2 stretching mode is questioned and that this mode may more approximately be assigned as the Fe^{II}–O–O bending mode (Benko & Yu, 1983).

The increase in ν(Fe–CO) frequency upon the replacement of *N*-MeIm by 1,2-Me_2Im becomes smaller when the Fe–C–O geometry is distorted, as observed in carbonmonoxy strapped hemes (E. A. Kerr, N.-T. Yu, and C. K. Chang, unpublished results), presumably because the above-mentioned σ effect becomes less important relative to the π effect (Yu et al., 1983). In this laboratory, the effect of Fe^{II}–N_ϵ (Im) tilting (with respect to the heme normal) on the ν(Fe–CO) frequency is also currently under investigation.

In order to confirm the ν(Fe–CO) frequency increase for constrained complexes, iron porphyrins other than picket fence were studied (Figure 5). For CO complexes of iron(II) tetraphenylporphyrin, Fe^{II}(TPP), the ν(Fe–CO) increases from 486 to 494 cm^{-1} (in C_6H_6). For CO complexes of iron(II) octaethylporphyrin, Fe^{II}(OEP), the ν(Fe–CO) increases from 496 to 501 cm^{-1} (also in C_6H_6). The slight differences observed between the octaethyl- and tetraarylporphyrins in the frequency increase for hindered complexes as well as the location of the ν(Fe–CO)'s may be due to the greater π-acceptor capacity of the tetraaryl- than octaethylporphyrins.

Mackin et al. (1983) studied the effect of proximal tension on the ν(Co–O_2) frequency in the O_2 complex of Co(II) picket fence porphyrins. As the O_2 binding affinity is lowered by a factor of 6.4 (Collman et al., 1978a) in going from the unstrained (*N*-MeIm) complex to the strained (1,2-Me_2Im) complex, the ν(Co–O_2) at 516 cm^{-1} for the high-affinity complex increases by 11 cm^{-1} to 527 cm^{-1} for the low-affinity complex. In cobalt complexes, the overlap between the imidazole π orbital and the π^* orbital of O_2 is not as extensive as in the case of iron complexes; hence, a decrease in π donation from 1,2-Me_2Im produces a relatively small decrease in π bonding between Co and O_2. Consequently, the major factor is the increase in σ bonding between Co and O_2 caused by the decrease in the Co–N_ϵ σ bonding.

Registry No. Fe^{III}(TpivPP)Br, 52215-70-0; Fe(TpivPP)(*N*-MeIm), 52215-89-1; Fe(TpivPP)(1,2-Me_2Im), 66200-05-3; Fe(TpivPP)(py), 86595-06-4; Fe(TpivPP)(THF), 52215-88-0; CO, 630-08-0; O_2, 7782-44-7; Fe^{III}(TPP)Br, 25482-27-3; Fe^{III}(OEP)Cl, 28755-93-3; Fe(TPP)(*N*-MeIm)(CO), 74902-03-7; Fe(TPP)(1,2-Me_2Im)(CO), 80975-58-2; Fe(OEP)(*N*-MeIm)(CO), 72918-56-0; Fe(OEP)(1,2-Me_2Im)(CO), 86584-34-1; Fe(TpivPP)(*N*-MeIm)(CO), 52215-85-7; Fe(TpivPP)(THF)(CO), 52215-86-8; Fe(TpivPP)(py)(CO), 86584-35-2; Fe(TpivPP)(1,2-Me_2Im)(CO), 86595-07-5; Fe(TpivPP)(CO), 86584-36-3; Fe(TpivPP)(*N*-MeIm)(O_2), 55449-22-4; Fe(TpivPP)-(1,2-Me_2Im)(O_2), 74171-97-4.

References

Alben, J. O., & Caughey, W. S. (1968) *Biochemistry 7*, 175–183.

Alben, J. O., Moh, P. P., Fiamingo, F. G., & Altschuld, R. A. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 234.

Baldwin, J. M. (1980) *J. Mol. Biol. 136*, 103.

Barlow, C. H., Ohlsson, P. I., & Paul, K. G. (1976) *Biochemistry 15*, 2225.

Benko, B., & Yu, N.-T. (1983) *Proc. Natl. Acad. Sci. U.S.A.* (in press).

Burke, J. M., Kincaid, J. R., Peters, S., Gagne, R. R., Collman, J. P., & Spiro, T. G. (1978) *J. Am. Chem. Soc. 100*, 6083–6088.

Caughey, W. S. (1970) *Ann. N.Y. Acad. Sci. 174*, 148.

Caughey, W. S. (1980) *Adv. Inorg. Biochem. 2*, 95.

Caughey, W. S., Alben, J. O., McCoy, S., Boyer, S. H., Charache, S., & Hathaway, P. (1969) *Biochemistry 8*, 59.

Caughey, W. S., Barlow, C. H., O'Keefe, D. H., & O'Toole, M. C. (1972) *Ann. N.Y. Acad. Sci. 206*, 296.

Caughey, W. S., Houtchens, R. A., Lanir, A., Maxwell, J. C., & Charache, S. (1978) in *Biochemical and Clinical Aspects of Hemoglobin Abnormalities* (Caughey, W. S., Ed.) pp 29–56, Academic Press, New York.

Chang, C. K., & Traylor, T. G. (1973) *J. Am. Chem. Soc. 95*, 8477.

Collman, J. P., Gagne, R. R., Reed, C. A., Halbert, T. R., Lang, G., & Robinson, W. T. (1975) *J. Am. Chem. Soc. 97*, 1427.

Collman, J. P., Brauman, J. I., Halbert, T. R., & Suslick, K. S. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 3333.

Collman, J. P., Brauman, J. I., Doxsee, K. M., Halbert, T. R., & Suslick, K. S. (1978a) *Proc. Natl. Acad. Sci. U.S.A. 75*, 564.

Collman, J. P., Brauman, J. I., Rose, E., & Suslick, K. S. (1978b) *Proc. Natl. Acad. Sci. U.S.A. 75*, 1052.

Collman, J. P., Brauman, J. I., & Doxsee, K. M. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 6035.

Goedken, V. T., Peng, S.-M., Molin-Norris, J., & Park, Y. A. (1976) *J. Am. Chem. Soc. 98*, 8391.

Heidner, E. J., Ladner, R. C., & Perutz, M. F. (1976) *J. Mol. Biol. 104*, 707.

Hoard, J. T., & Scheidt, W. R. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 3919.

Hori, H., & Kitagawa, T. (1980) *J. Am. Chem. Soc. 102*, 3608.

Huber, R., Epp, O., & Formanek, H. (1970) *J. Mol. Biol. 52*, 349.

Kerr, E. A., Mackin, H., & Yu, N.-T. (1982) *Biophys. J. 37*, 371a.

Lesk, A. M. (1982) *Introduction to Physical Chemistry*, pp 350–352, Prentice-Hall, Englewood Cliffs, NJ.

Mackin, H. C., Tsubaki, M., & Yu, N.-T. (1983) *Biophys. J. 41*, 349–357.

Makinen, M. W., Houtchens, R. A., & Caughey, W. S. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 6042.

Norvell, J. C., Nunes, A. C., & Schoenborn, B. P. (1975) *Science (Washington, D.C.) 190*, 568.

O'Keefe, D. H., Ebel, R. E., Peterson, J. A., Maxwell, J. C., & Caughey, W. S. (1978) *Biochemistry 17*, 5845.

Padlan, E. A., & Love, W. E. (1975) *J. Biol. Chem. 249*, 4067.

Peng, S. M., & Ibers, J. A. (1976) *J. Am. Chem. Soc. 98*, 8032.

Perutz, M. F. (1970) *Nature (London) 228*, 726.

Perutz, M. F. (1976) *Br. Med. Bull. 32*, 195.

Rougee, M., & Brault, D. (1975) *Biochemistry 14*, 4100.

Scheidt, W. R., Haller, K. J., Fons, M., Mashiko, T., & Reed, C. A. (1981) *Biochemistry 20*, 3653.

Steigemann, W., & Weber, E. (1979) *J. Mol. Biol. 127*, 309.

Tsubaki, M., Srivastava, R. B., & Yu, N.-T. (1982) *Biochemistry 21*, 1132.

Walters, M. A., Spiro, T. G., Suslick, K. S., & Collman, J. P. (1980) *J. Am. Chem. Soc. 102*, 6857.

Wayland, B. B., Mehne, L. F., & Schwartz, J. (1978) *J. Am. Chem. Soc. 100*, 2379.

Yoshikawa, S., Choc, M. G., O'Toole, C., & Caughey, W. S. (1977) *J. Biol. Chem. 252*, 5498.

Yu, N.-T., & Srivastava, R. B. (1980) *J. Raman Spectrosc. 9*, 166.

Yu, N.-T., Kerr, E. A., Ward, B., & Chang, C. K. (1983) *Biochemistry* (paper in this issue).