RESONANCE RAMAN STUDIES OF DIOXYGEN AND CARBON MONOXIDE BINDING TO IMIDAZOLE-APPENDED HEMES

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ABSTRACT Resonance Raman spectroscopy has been employed to probe the effects of proximal base strain on the bonding of O_2 and CO in three synthetic hemins with covalently linked imidazole ligands. The strain is introduced by varying the length of the imidazole-containing side chain and by restricting the side chain flexibility with a phenyl ring. These hemins are abbreviated as "long," "short," and "stiff" hemins, respectively. In the deoxy state, the iron-imidazole stretching frequencies [ν(Fe—N_ϵ)] for long, short, and stiff hemins are detected at 200, 207, and 204 cm^{-1}, respectively. The strain induced in the iron–imidazole bond by the short hemin results in a higher ν(Fe—N_ϵ) frequency, in contrast to the strain induced by sterically hindered 2-methylimidazole or 1,2-dimethylimidazole complexes in which the Fe—N_ϵ bond is tilted and lengthened, but the imidazole ring remains perpendicular to the heme plane. However, in the short hemin, the plane of the imidazole ring may not be perpendicular to the plane of the porphyrin, altering the amount of π-interaction (hence the strength of Fe—N_ϵ bond) and the nature of normal mode containing Fe—N_ϵ bond stretching. Upon CO binding, we have observed the ν(Fe—CO) stretching frequencies at 497 (long), 499 (short), and 496 cm^{-1} (stiff), somewhat lower than those reported by Mitchell et al. (*Inorg. Chem.*, 1985, 24:967) for the chelated-heme · CO complexes (i.e., 501–506 cm^{-1}). This is the first report of an iron–oxygen-associated vibration observed in solution for an unprotected heme. The oxy complexes were formed by introducing dioxygen to the deoxy complexes at −70°C. The isotope-sensitive line was detected at 576 cm^{-1} ($^{16}O_2$) in oxy stiff hemin, which was shifted to 545 cm^{-1} upon $^{18}O_2$ substitution. This is perhaps the largest isotope shift (31 cm^{-1}) observed to date, compared with the usual 22–24 cm^{-1}. For the long and short hemins, the iron–oxygen-associated vibration was detected at 574 and 573 cm^{-1}, respectively. These values are very similar to those observed in the oxy complexes of iron(II) "picket-fence" porphyrin · (*N*-methylimidazole) and myoglobin/hemoglobin.

INTRODUCTION

An important contribution to the study of hemoproteins has been the synthesis and detailed investigation of a variety of specifically designed model porphyrin complexes. Different heme models (1–8) have been made to mimic specific features and functions of the protein systems. The bond that exists between the iron and proximal imidazole in the fifth ligand position is thought to be important in controlling heme reactivity. The tension or lack of tension in that bond is believed to be related to the affinity of human hemoglobin for oxygen, and may play an important role in cooperative oxygen binding (9). Model compounds that specifically probe this proximal base tension are useful in investigating this effect. In this study, specially synthesized porphyrins with covalently linked imidazole bases were employed. These compounds, whose structures are shown in Fig. 1, differ by the length and nature of the linkage connecting the porphyrin side group to the imidazole, which serves as the fifth ligand to the iron. These three compounds are termed "long," "short," and "stiff" hemins. Recently, Mitchell et al. (10) studied chelated heme compounds in which an imidazole is covalently bound to a side chain attached to the β-position of a mesoheme. However, the chelated hemes employed in our present study are derived from meso-substituted diphenyletioporphyrins. The imidazole-containing side chain is connected to the ortho-position of a *meso*-phenyl ring, the rotation of which is restricted. Thus, the imidazole coordination to the heme iron is enforced (11).

Resonance Raman spectroscopy is employed to investigate the effect of molecular strain caused by shortening the chain length. Comparison of the iron–imidazole stretching vibrations of the deoxy complexes, the iron–carbon stretching frequencies in the carbonmonoxy complexes, and the iron–oxygen frequencies in the oxy complexes can lead to a better understanding of the proximal base tension effect. We report for the first time the iron–oxygen vibration in the resonance Raman spectra of oxy complexes of iron(II) porphyrin without a cavity such as picket fence porphyrin.

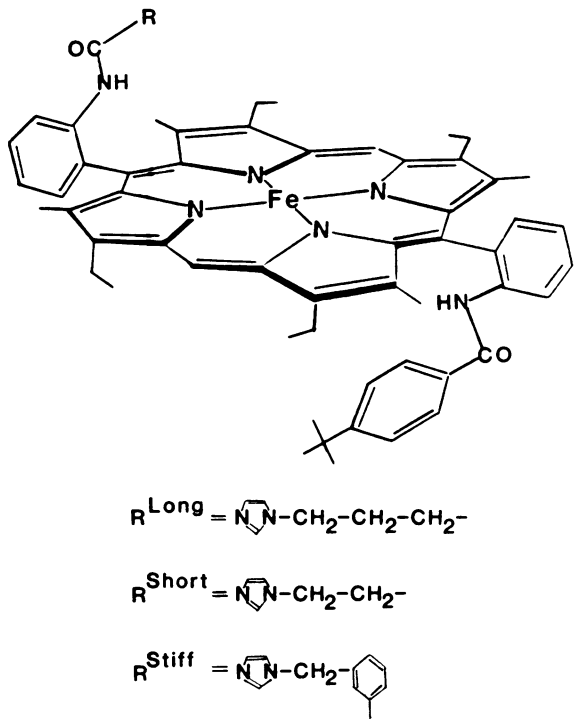

FIGURE 1 Chemical structure of long, short, and stiff hemins.

EXPERIMENTAL METHODS

The three imidazole-appended *meso*-diphenyletioporphyrins (Fig. 1) were synthesized by the method of Young and Chang (11). For the resonance Raman experiments, the hemins were prepared first as the carbonmonoxy complex, then deoxy, and then the oxy complex. To prepare the carbonmonoxy complex, a ~80–100-μM solution of hemin in benzene was degassed by evacuation and flushed with carbonmonoxide (Matheson Gas Products, Inc., Seacaucus, NJ). A few drops of a 10% Vitride T reducing agent (70% $C_6H_{16}AlNaO_4$ in toluene; J. T. Baker Chemical Co., Phillipsburg, NJ) solution in benzene were added to reduce the iron and allow formation of the carbonmonoxy complex.

The deoxy complexes were formed from the carbonmonoxy complexes. The Raman cell containing the carbonmonoxy complex was evacuated and filled with prepurified nitrogen gas (99%; Matheson Gas Products, Inc.). The Raman spectra were then obtained, but without spinning of the Raman cell. This allowed any remaining bound carbonmonoxide to be photolyzed off by the laser light. After obtaining the spectra, spinning of the sample was resumed, allowing rebinding of the carbonmonoxide. Regeneration of the carbonmonoxide spectrum was evidence that the sample did not undergo appreciable oxidation.

To obtain the resonance Raman spectra of the oxy complexes, a special low-temperature apparatus (12) was employed. The samples were prepared first as the carbonmonoxy complexes, then as the deoxy forms. However, methylene chloride was used as the solvent in order to reach the low temperature in liquid solution state. After the deoxy complex was cooled to -70°C (monitored by a microprocessor-based thermocouple; model AD 2050, Cole-Parmer Instrument Co., Chicago, IL), oxygen gas was then flushed through the rubber septum on the Raman cell. Spinning of the sample cell was resumed and the oxy spectra were obtained.

Obtaining the $^{18}O_2$-oxy spectrum was slightly more difficult, since $^{18}O_2$ could not be flushed through the sample. Hence, a vacuum line was connected to the sample cell and used to evacuate the Raman cell which was still in the low-temperature apparatus. The $^{18}O_2$ gas (99% ^{18}O, Prochem Isotopes, US Services Inc., Summit, NJ) was then introduced via a needle through the rubber septum.

All spectra were obtained with a multichannel laser Raman system as described by Yu and Srivastava (13). The excitation wavelengths at 406.7 and 413.1 nm were provided by a Krypton ion laser (model 171, Spectra-Physics Inc., Mountain View, CA).

RESULTS

The resonance Raman spectra of the carbonmonoxy complexes of long, short, and stiff hemins, excited at 413.1 nm, are shown in Fig. 2. The strong lines at 497, 499, and 496 cm^{-1} of long, short, and stiff hemins, respectively, are assigned as the iron–carbon stretching mode, ν(Fe—CO). These values are similar to the ν(Fe—CO) of iron(II)-octaethylporphyrin (*N*-methylimidazole)CO (14) at 496 cm^{-1} and the ν(Fe—CO) of iron(II) tetraphenylporphyrin(*N*-methylimidazole)CO (14) at 486 cm^{-1}. The chelated-heme · CO complexes studied by Mitchell et al. (10) exhibit a somewhat higher ν(Fe—CO) frequency, i.e., 501–506 cm^{-1}. The long hemin, which presumably has a perpendicular Fe—N_ϵ bond, gave rise to a ν(Fe—CO) ~3 cm^{-1} lower than the short hemin (with a tilted Fe—N_ϵ bond). This is consistent with another study investigating proximal base effects (14) where Fe(II)(TpivPP)(1,2-Me_2Im)CO gave rise to a ν(Fe—CO) 7 cm^{-1} higher than the sterically unhindered *N*-methylimi-

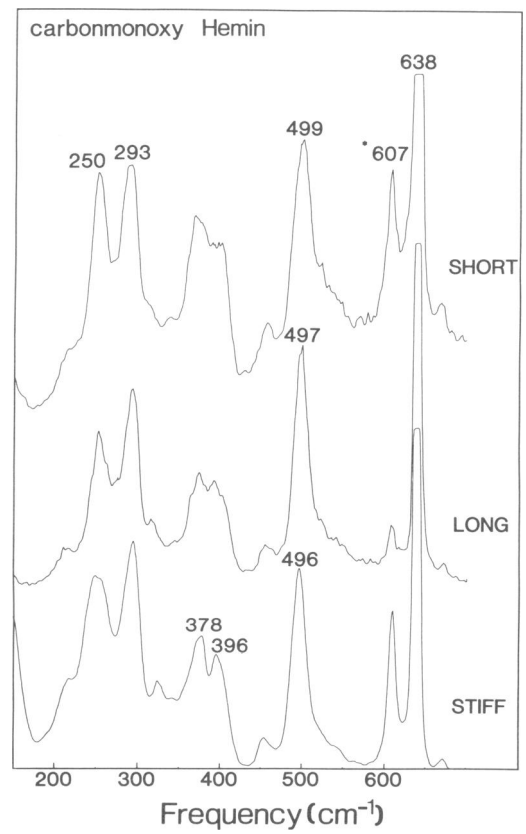

FIGURE 2 Resonance Raman spectra of carbonmonoxy hemins in benzene. Excitation wavelength (λ_{exc}), 413.1 nm; laser power, 20 mW; heme concentration, 100 μM. The symbol (*) denotes benzene lines.

dazole complex. This has been explained (14) in terms of relative σ and π contributions of the imidazole base and its effect on the iron–carbon bond.

Fig. 3 shows the resonance Raman spectra of the deoxy complexes of long, short, and stiff hemins. A new line at ~200 cm^{-1} is present in all three complexes that is not present in the carbonmonoxy or oxidized forms. This is assigned to the iron–imidazole stretching frequency on the basis of similar assignments in the deoxy complexes of hemoproteins and other metalloporphyrins (15–19). Similar lines were observed in the resonance Raman spectra of deoxy iron(II) picket-fence porphyrins (15). For the sterically hindered 2-methylimidazole complex, the iron–imidazole stretching frequency was identified at 209 cm^{-1} and confirmed with ^{54}Fe and deuterated 2-methylimidazole isotope studies. The *N*-methylimidazole complex gave rise to a line at 225 cm^{-1}, but was weak and not confirmed by isotope studies. This complex also presents the problem that six-coordination is possible with *N*-methylimidazole. In the case of deoxy Fe(II) adamantane porphyrin-6,6-cyclophane (APC) with N-MeIm as the axial base (Yu, N. -T., E. A. Kerr, and T. G. Traylor, unpublished results) (20) observed a strong iron–imidazole stretching vibration at 214 cm^{-1} upon excitation at 406.7 nm. In deoxy T-state hemoglobin, the iron–imidazole stretching vibration was observed at 216 cm^{-1}, which shifted to 220 cm^{-1} in R-state deoxy hemoglobin (21).

Long, short, and stiff hemins gave rise to slightly different values of the iron–imidazole stretching frequency: 200 cm^{-1} (long), 207 cm^{-1} (short), and 204 cm^{-1} (stiff). The frequency at 204 cm^{-1} is uncertain because of the steep baseline. Given a flat baseline, it should appear at a frequency somewhat higher than the 204 cm^{-1} value indicated here.

In Fig. 4, the oxy complexes of long, short, and stiff hemins are presented. In the upper panel, oxy stiff hemin with $^{16}O_2$ and $^{18}O_2$ are shown. The line at 576 cm^{-1} shifts to 545 cm^{-1} with $^{18}O_2$. The lower panel shows the resonance Raman spectra of oxy long and short hemins, where the iron–oxygen vibrations appear at 574 and 573 cm^{-1}, respectively.

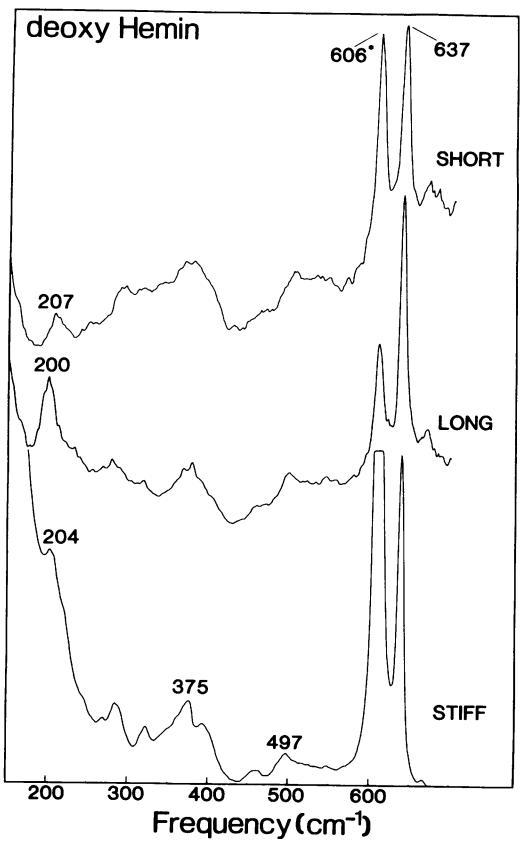

FIGURE 3 Resonance Raman spectra of deoxy hemins in benzene. Experimental conditions same as in Fig. 2. The symbol (*) denotes benzene line.

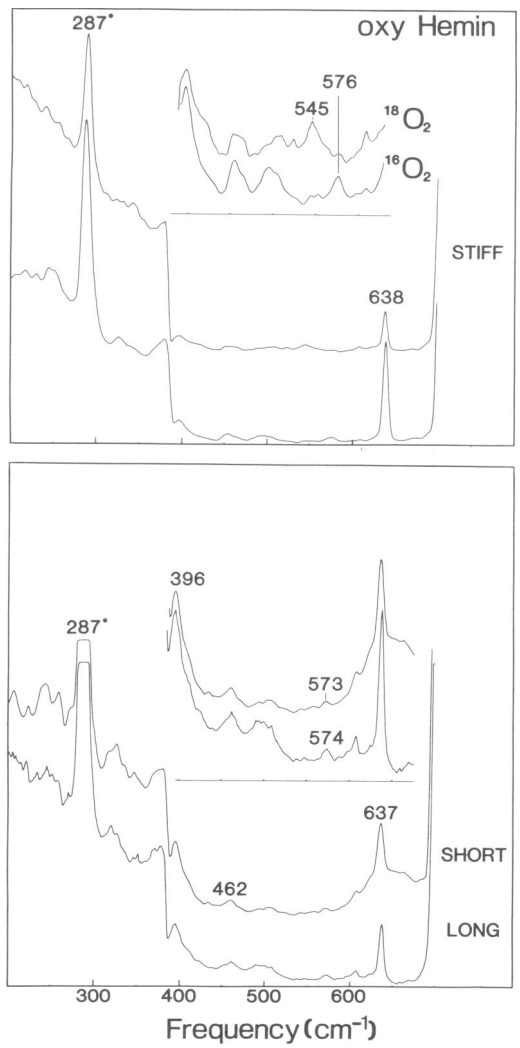

FIGURE 4 Resonance Raman spectra of oxy hemins in methylene chloride; $T = -70°C$. (*Top*) Oxy stiff hemin, $^{16}O_2$ and $^{18}O_2$ complexes. (*Bottom*) Long and short hemins, $^{16}O_2$ complexes. Experimental conditions same as in Fig. 2.

DISCUSSION

Deoxy Hemins

For the deoxy complexes, the long hemin gave rise to a lower iron–imidazole stretching frequency than the short hemin. The stiff hemin gave rise to an intermediate value. This is not consistent with the results of the deoxy "picket fence" study (15). In that study, the more sterically hindered axial base gave rise to a lower iron–imidazole stretching vibration than the unhindered *N*-methylimidazole complex. This behavior was also observed in another model complex, iron(II) "pocket" porphyrin Fe(II)(Poc-Piv) (5). With the sterically hindered 2-methylimidazole complex, the ν(Fe—N_ϵ) was observed at 215 cm^{-1}, while the *N*-methylimidazole complex gave rise to a ν(Fe—N_ϵ) at 227 cm^{-1}.

It is possible that two different types of strain, or steric hindrance, exist between the "picket-fence" complexes and these long, short, and stiff hemins. For the picket-fence complexes, replacement of *N*-methylimidazole with either 2-methyl or 1,2-dimethylimidazole results in a "face strain" (22) in which the Fe—N_ϵ bond is lengthened and tilted from the heme normal. However, the imidazole ring is still perpendicular to the heme plane. Thus the major effect is the weakening of the Fe—N_ϵ bond, resulting in a lower iron–imidazole stretching frequency. In contrast, shortening the chain length (from long hemin to short hemin) causes not only the weakening of the Fe—N_ϵ bond, but also the inclination of the imidazole so that its plane is no longer perpendicular to the porphyrin plane. It is interesting that the νFe—N_ϵ) frequency increases from 200 cm^{-1} (long hemin) to 207 cm^{-1} (short hemin). Normally, the weakening of the Fe—N_ϵ bond is expected to cause a decrease in the Fe—N_ϵ stretching frequency. To account for the frequency increase, one should carry out normal coordinate calculations comparing two types of imidazole ring orientations. In the Fe—N_ϵ stretching mode, all the atoms in the imidazole ring move more or less in-phase; the ν(Fe—N_ϵ) frequency depends on the effective mass of the imidazole. This is analogous to the Fe—C—O moiety, where the ν(Fe—C) stretching frequency increases as the Fe—C—O bond angle decrease (23, 24). This is because the effective mass of CO for Fe—C stretching vibration decreases as the Fe—C—O bond angle decreases. In extreme case where the Fe—C—O angle is 90°, oxygen mass make no contribution to the effective mass of CO. Thus, it is conceivable that the frequency increases in ν(Fe—N_ϵ) from long hemin to short hemin could be caused by a lowering of the effective mass of the imidazole because of its inclination. The evidence for the dependency of the ν(Fe—N_ϵ) frequency on imidazole mass comes from resonance Raman study of iron(II) adamantane with *N*-methylimidazole [ν(Fe—N_ϵ) = 214 cm^{-1}] and the heavier *N*-triphenylmethylimidazole [ν(Fe—N_ϵ) = 203 cm^{-1}] complexes (19). Both axial bases cause no steric hindrance but do differ in mass. Since the two effects, weakening of the Fe—N_ϵ bond and the decrease in effective reduced mass, cause the ν(Fe—N_ϵ) frequency to shift in opposite directions, it is possible that these effects may cancel each other producing no change in the ν(Fe—N_ϵ) frequency. This may be the case reported by Mitchell et al. (10) who detected no difference in the ν(Fe—N_ϵ) frequency (at 204 cm^{-1}) in chelated hemins with different chain lengths.

The important consideration, however, is that the results of this study do not correlate with the deoxy R and T state hemoglobin results (21). In other words, the strain imposed on the iron–imidazole bond in short hemin relative to long hemin does not have the same effect on ν(Fe—N_ϵ) as the difference in strain that most likely exists between R and T state deoxy hemoglobin.

Oxy Hemins

The oxy complexes of these hemins are of interest for one important reason. It is the first identification of an iron–oxygen vibration in the resonance Raman spectrum of an unprotected, oxy iron(II) porphyrin in solution. Usually, an iron(II) porphyrin will be oxidized to an iron(III) species in the presence of oxygen. This is believed to proceed through the formation of a μ-oxo species, facilitated by close approach of two iron centers (25). However, in this study, the oxy complexes were stabilized by cooling the deoxy complexes to −70°C before the addition of oxygen, as was demonstrated previously with a similar chelated hemin (26).

In Fig. 4 (*top*), the oxy stiff hemin with $^{16}O_2$ and $^{18}O_2$ shows that the line at 576 cm^{-1} shifts to 545 cm^{-1} with $^{18}O_2$. This is assigned as a vibration associated with the iron–oxygen bond. It is not certain whether this vibration is best classified as the iron–oxygen stretching or bending vibration (27). Also of interest is that this is the largest isotope shift observed to date, 31 cm^{-1}, instead of the usual 22–24 cm^{-1}. For the long and short hemins, this vibration is located at 574 and 573 cm^{-1}, respectively. This vibration is nearly insensitive to the length or nature of the imidazole–porphyrin linkage.

Surprisingly, the isotope-sensitive lines in the three hemin complexes were fairly similar to the values observed (14) in Fe(II)(TpivPP)(N-MeIm)O_2 at 571 cm^{-1} and oxyhemoglobin A at 570 cm^{-1}. Thus, in these very different environments, the iron–oxygen vibration remains fairly constant. The insensitivity of this vibration to its local environment is different from what is observed in oxy cobalt hemes or carbonmonoxy hemes. The ν(Fe—CO) is different in picket-fence complexes [ν(Fe—CO) = 489 cm^{-1} for Fe(TpivPP)(N-MeIm)CO (14), hemoproteins [ν(Fe—CO) = 512 cm^{-1} for MbCO] (28), and unprotected carbonmonoxy heme complexes [ν(Fe—CO) = 486 cm^{-1} in Fe(TPP)(N-MeIm)CO/benzene] (14). The iron-carbon bond could be affected by steric restraints in the protein or solvent effects due to the polar nature of carbonmonoxide. In oxy cobalt hemes, the additional elec-

tron in cobalt, which resides primarily in the π^* orbital of oxygen, may be more sensitive to different environments. The cobalt–oxygen linkage may be stabilized in environments that favor the $Co^{\delta+} - O_2^{\delta-}$ formation, such as polar solvents or hydrogen bond formation in the heme pocket. The $\nu(Co—O_2)$ stretching vibration (29) in heme models appears at ~516 cm^{-1} and in cobalt-substituted hemoproteins at ~540 cm^{-1}.

The iron–oxygen vibration has been found to be sensitive to axial base tension (14). For Fe(II)(TpivPP)(L)O_2, in benzene when L is *N*-methylimidazole, the iron–oxygen vibration is 571 cm^{-1} and when L is 1,2-dimethylimidazole, it is at 562 cm^{-1}. However, in these long, short, and stiff hemins the iron–oxygen frequencies are nearly insensitive to the length or nature of imidazole–porphyrin linkages.

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