Resonance Raman enhancement of the Mn—N—O bending mode in nitrosyl manganese "strapped" and "open" heme complexes

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ABSTRACT Resonance Raman spectra of the Mn^{II}—NO moiety in synthetic nitrosyl manganese heme complexes with and without steric hindrance are reported. The "strapped" hemes having a hydrocarbon strap (variable length) across one face of the heme hinder the perpendicular bonding of a linear ligand. These complexes were employed to investigate the effects of ligand distortion (primarily tilting) on Mn—NO stretching, Mn—N—O bending, and N—O stretching modes. It is demonstrated that ligand distortion in the Mn^{II}—NO system is a valid mechanism for causing the resonance enhancement of the Mn—N—O bending mode, similar to that observed in the Fe^{II}—CO system (Yu, N.-T., E. A. Kerr, B. Ward, and C. K. Chang. 1983. *Biochemistry.* 22:4534–4540). More interesting is the observation of the δ(Mn—N—O) enhancement caused by the tilting of the *trans* Mn—N_ϵ bond in the "open" heme complexes (e.g., heme-5 and proto-1X dimethylester) with 1,2-dimethylimidazole or piperidine as a base. The ν(Mn—NO) and ν(N—O) modes exhibit an increase and a decrease, respectively, as the strap length decreases (hence the steric hindrance increases). Both ν(Mn—NO) and ν(N—O) frequencies are insensitive to the strength of the *trans* base. The results from "strapped" and "open" model heme systems imply that the Mn—N—O geometry is essentially linear and perpendicular in the nitrosyl complexes of monomeric manganese insect hemoglobin CTT IV and sperm whale myoglobin. The unusually low ν(N—O) frequency in the manganese myoglobin complex may be caused by the distal histidine—NO interaction. The δ(Mn—N—O) enhancement in both nitrosyl manganese CTT IV and nitrosyl manganese myoglobin may be caused by a tilting of the Mn^{II}—N_ϵ (proximal histidine) bond.

INTRODUCTION

The "strapped" heme (1) which sterically hinder the binding of a ligand provide excellent model systems for investigating the effects of ligand distortion on the vibrational modes (hence the bonding interactions) associated with a linear M—AB moiety (M = metal, AB = diatomic ligand) in the heme complexes. These synthetic hemes have a covalently linked hydrocarbon strap (13-, 14- or 15-atoms long) across one face of the heme providing a sideway shearing strain to force the linear ligand to be tilted and/or to be bent (2). Previously, it was demonstrated that the binding affinity of carbon monoxide decreases with decreasing strap length, presumably because of the increasing steric hindrance (1). Yu et al. (2) studied the resonance Raman spectra of the heme—CO complexes with and without the molecular strap. These authors found that Fe—CO stretching frequency increases and the C—O stretching frequency decreases as the strap length is decreased. This is somewhat surprising in view of the expected weakening of the Fe—CO bond associated with the off-axis bonding and the accompanying decrease in π backbonding.

It is most interesting to observe that the carbonmonoxy "strapped" hemes exhibit a Fe—C—O bending mode, whereas the open "unstrapped" hemes complexed with CO do not. It appears that ligand distortion is the most plausible mechanism for causing the resonance enhancement of the Fe—C—O bending mode. It was proposed that the Fe—C—O distortion (primarily tilting with a small bend) increases the overlap between CO (π^*) and porphyrin (π^*) orbitals, which provide direct coupling of the Fe—C—O bending mode with the resonant Soret ($\pi - \pi^*$) transition (2). No other enhancement mechanism has been found for the heme—CO systems. Therefore, the observed enhancement of the Fe—C—O bending mode in carbonmonoxy hemoglobins is an indicator of the Fe—C—O distortion.

In this paper we report resonance Raman studies of nitrosyl manganese "strapped" and "open" hemes having a Mn^{II}—NO moiety, isoelectronic with Fe^{II}—CO moiety. Specifically, we have investigated: (*a*) mechanisms for the resonance enhancement of the Mn—N—O bending mode, (*b*) the effects of ligand distortion on Mn—NO stretching and N—O stretching modes, and (*c*) the *trans* effects between the Mn^{II}-base and Mn^{II}—NO bonds. The results obtained from these model heme systems are then employed to interpret resonance Raman spectra of the nitrosyl complexes of monomeric manganese hemoproteins, such as insect hemoglobin (CTT IV) (3) and sperm whale myoglobin (Mb). Previously, Parthasarathi and Spiro (4) reported the first assignments of ν(Mn—NO)

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and δ(Mn—N—O) modes, and discussed bonding differences with respect to Fe—CO, and protein differences with respect to a model complex.

We demonstrate that ligand distortion in the Mn^{II}—NO system is a valid mechanism for the enhancement of the δ(Mn—N—O) mode. Furthermore, the distortion of the Mn^{II}-base bond by the 2-methyl group of 1,2-dimethylimidazole base can also cause the resonance Raman enhancement of the bending mode. This is a fundamental difference from the above mentioned Fe^{II}—CO heme system. In addition, we offer an explanation for the previously reported enhancement of the Mn—N—O bending mode in the Mn^{II} (proto-IX) (piperidine) complex in neat piperidine (4).

The analysis of our spectral data suggests that the Mn—N—O moiety in CTT IV and Mb is essentially linear and perpendicular to the heme plane. The observed Mn—N—O bending mode enhancement may be caused by the distortion of the Mn—N_ϵ (proximal histidine) bond in CTT IV and Mb, in analogy to the enhancement in "open" heme complexes with 1,2-dimethylimidazole.

MATERIALS AND METHODS

Preparation of samples

The manganese "strapped" hemes (Mn SP-13, Mn SP-14, and Mn SP-15) and manganese heme-5 were synthesized by the method described previously (1). Manganese proto-IX-DME (protoheme-IX dimethyl ester) and manganese meso-IX-DME were obtained from Midcentury, Inc. (Posen, IL) and were used without further purification. The oxidation state in all compounds was Mn^{III}.

The heme benzene solution (1.0 ml, 100 μM heme with 100-fold base in benzene) were reduced by adding a minimum amount of Red-Al^R reducing agent (3.4 M $[(CH_3OCH_2CH_2O)_2AlH_2]Na$). The reduced heme benzene solution was then transferred by a syringe into an oxygen-free Raman cell which had been filled previously with nitric oxide gas.

Most reagents used were purified and stored under N_2. Benzene was stirred over concentrated H_2SO_4 to remove organic impurities, neutralized with a dilute $NaHCO_3$ solution, dried over anhydrous $MgSO_4$, and distilled from sodium metal. *N*-Methylimidazole (Sigma Chemical Co., St. Louis, MO) was distilled from KOH, and 1,2-dimethylimidazole (Aldrich Chemical, Milwaukee, WI) was distilled from Na and recrystallized from benzene. Piperidine (Aldrich Chemical Co.) was used without further purification.

Purification and reconstitution of manganese CTT IV

The monomeric hemoglobin CTT IV from *Chironomus thummi thummi* was purified as previously described (5, 6). The salt-free lyophilized protein was stored at −30°C in the met form. Mn-protoheme-IX and Mn-mesoheme-IX were prepared using standard methods (7). The preparation of globin and the reconstitution of CTT IV with Mn-protoheme-IX and Mn-mesoheme-IX were carried out employing the methods described elsewhere (8, 9). The oxidized salt-free manganese CTT IV was lyophilized and stored at −30°C.

Preparation of nitrosyl complexes of manganese CTT IV

The manganese CTT IV solutions (~100 μM) were prepared by dissolving the lyophilized oxidized hemoglobin in the appropriate buffers; these buffers were either 0.2 M citrate/phosphate (pH 5.1), 0.2 M Tris/HCl (pH 9.4), and 0.1 M phosphate buffer (pH 7.0). Undissolved material was completely removed by centrifugation. The nitrosyl CTT IV complexes were prepared as follows. A very small amount of sodium dithionite (~0.5 mg) or ascorbic acid was placed in a cylindrical quartz Raman cell. After the cell was sealed and evacuated, nitric oxide gas ($^{14}N^{16}O$, Matheson Gas Products, Secaucus, NJ; $^{15}N^{16}O$, 99 atom % ^{15}N, Prochem US Services, Inc., Summit, NJ) was introduced into the cell. Then the degassed manganese hemoglobin solution was added via a pressure-lock syringe. The complexes with $^{14}N^{18}O$ isotope were prepared in a similar manner except that $^{14}N^{18}O$ was generated by reducing potassium nitrite ($K^{14}N^{18}O_2$, 90 atom % ^{18}O, Stohler Isotope Chemicals, Waltham, MA) with ascorbic acid in the upper chamber of the Raman cell.

RESONANCE RAMAN SPECTROSCOPY

All Raman spectra were recorded with the highly sensitive multichannel laser Raman system (10), which consists of a double monochromater (model 1402, Spex Industries, Inc., Edison, NJ) equipped with a dry-ice cooled SIT vidicon detector (model 1254, Princeton Applied Research, Princeton, NJ), a detector controller (model 1216, Princeton Applied Research), and OMA 3 console (model 1260-V, Princeton Applied Research). The 406.7 and 413.1 nm lines of a krypton ion laser (model 171-01, Spectra-Physics, Inc., Mountain View, CA) were employed as excitation sources. Power was ~15 mW at the sample. Scattered light was collected at a 90° geometry and the entrance slit was opened 100 μm in width and 0.2 cm in height. The sample was spun throughout the measurements to eliminate photodissociation. All spectra were collected at room temperature. No smoothing procedures were employed. Calibrations were performed with standard compounds. The reported wave numbers are accurate within ±1 cm^{-1} for sharp peaks and ±2 cm^{-1} for broad lines.

RESULTS

Chemical structure of model compounds

Fig. 1 shows the chemical structures of manganese heme-5 "open" (or "unstrapped") and manganese "strapped" hemes with various chain lengths (SP-15, SP-14, and SP-13), complexed with nitrosyl and *N*-methylimidazole as axial ligands.

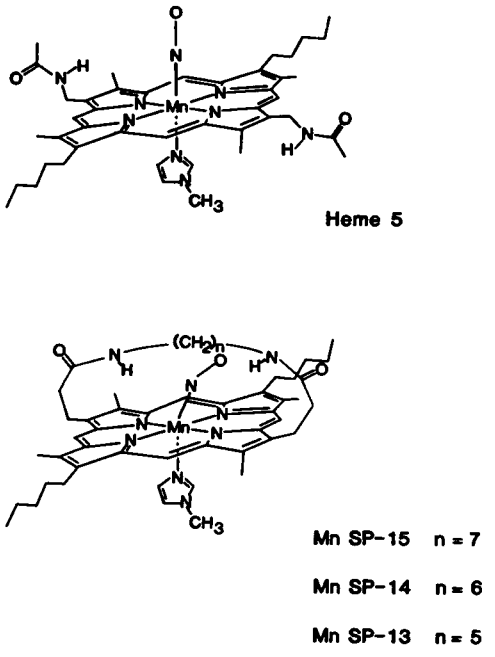

FIGURE 1. Chemical structures of manganese heme-5 and manganese "strapped" hemes.

Identification of nitrosyl manganese vibrational modes

The Mn—NO stretching, Mn—N—O bending, and N—O stretching modes in monomeric nitrosyl manganese insect hemoglobin (CTT IV) have been clearly identified by ligand isotope substitution (with $^{14}N^{16}O$, $^{15}N^{16}O$, and $^{14}N^{18}O$) at 628 cm^{-1}, 574 cm^{-1}, and 1,736 cm^{-1}, respectively (see Table 1) (3). Substitution of protoporphyrin-IX by mesoporphyrin-IX does not affect these frequencies.

The nitrosyl complexes of "open" manganese porphyrin systems without steric hindrance (heme-5 and PPDME) and with *N*-methylimidazole as the fifth ligand exhibit Raman lines at 629 (±1) cm^{-1} and 1,734 (±2) cm^{-1} which can be assigned with confidence to Mn—NO stretching and N—O stretching vibrations by analogy with the respective nitrosyl complexes of manganese CTT IV. The Mn—N—O bending mode in these "open" heme model complexes is not enhanced. Even with various Soret excitation lines (406.7, 413.1, and 415.4 nm) no enhanced Mn—N—O bending mode could be observed. This finding is not surprising for complexes with linear and perpendicular Mn—N—O geometry.

The Mn—N—O bending mode of the "open" heme-5 and proto-IX DME complexes is enhanced when the axial *trans* ligand is 1,2-dimethylimidazole or piperidine. In the case of 1,2-dimethylimidazole, the heme-5 complex exhibits its Mn—N—O bending mode at 574 cm^{-1} (Fig. 2 *A*, curve *b*), whereas the proto-IX DME complex shows the Mn—N—O bending mode at 573 cm^{-1} (Fig. 2 *B*, curve *b*). In the case of piperidine, the Mn—N—O bending mode appears at 570 cm^{-1} for the heme-5 complex (Fig. 2 *A*, curve *c*) and at 568 cm^{-1} for the proto-IX DME complex (Fig. 2 *B*, curve *c*). Previously, this enhanced bending mode was observed for the Mn^{II} (proto-IX DME) (piperidine) (NO) complex dissolved in neat piperidine (4). We have repeated the experiments and verified this observation.

The preparation of the nitrosyl Mn^{II} (proto-IX DME) complex with *N*-methylimidazole as a base failed in methanolic solution (4). However, we found that this complex can be formed in benzene solution (see Fig. 2 *B* [*a*]).

TABLE 1 Resonance Raman vibrational modes of nitrosyl manganese heme complexes

Heme base	δ[Mn-N-O]	ν[Mn-NO]	ν[N-O]
$^{14}N16_O$ $^{15}N16_O$	$^{14}N16_O$ $^{15}N16_O$	$^{14}N16_O$ $^{15}N16_O$	$^{14}N16_O$ $^{15}N16_O$
Proto-IX CTT IV*	574 (563)	628 (625)	1736 (1701)
Meso-IX CTT IV*	573 (562)	628 (623)	1735 (1698)
Proto-IX Mb‡	574 (558)	627 (621)	1713 (1677)
Heme-5 N-MeIm	NE	629	1736
Heme-5 1,2-Me_2Im	570 (568)	630 (626)	1736 (1699)
Heme-5 Pip	570 (560)	630 (627)	1738 (1701)
SP-15 N-MeIm	578	631	1727
SP-15 Pip	572 (561)	632 (629)	1727 (NM)
SP-14 N-MeIm	575	632	1718
SP-14 1,2-Me_2Im	573	631	NE
SP-14 Pip	571	634	1716
PPDME N-MeIm	NE	628	1733
PPDME 1,2-Me_2Im	573	630	1735
PPDME Pip	572 (weak)	629	1737
PPDME neat Pip	568	629	1739
PPDME neat Pip‡	566 (557)	627 (624)	1735 (1700)

NE, no enhanced resonance; NM, no measurement. *Reference 3; ‡reference 4.

RESONANCE RAMAN SPECTRA OF NITROSYL MANGANESE "STRAPPED" HEME COMPLEXES

Three different "strapped" hemes were used in this study which differ in the chain length of the strap. The axial base in these complexes was *N*-methylimidazole. Because Mn^{II} SP-13 failed to form a nitrosyl complex, we report only data for Mn^{II} SP-14 and Mn^{II} SP-15. Both complexes exhibit enhanced Mn—N—O bending modes at 575 cm^{-1} and 578 cm^{-1}, respectively (Fig. 3). The Mn—NO stretching frequency in these "strapped"

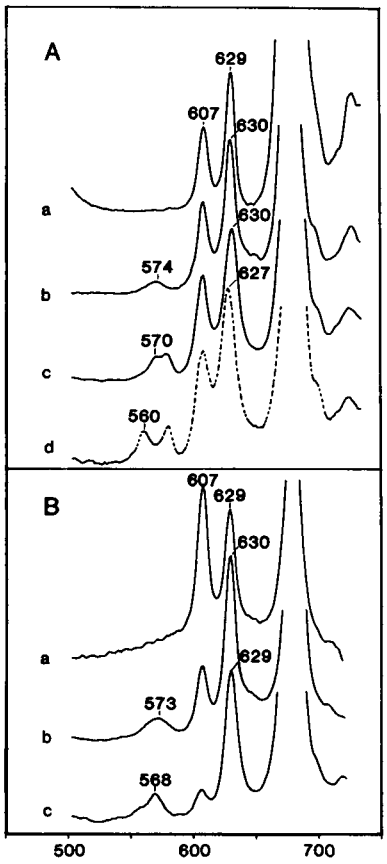

FIGURE 2. Resonance Raman spectra: (*A*) Mn^{II} (heme-5) (X) (NO) in benzene where X = *N*-methylimidazole (*a*), 1,2-dimethylimidazole (*b*), and piperidine (*c*). Spectrum *d* is the same as *c* except for the substitution of NO by ^{15}NO. (*B*) Mn^{II} (PPDME) (X) (NO) where X = *N*-methylimidazole (*a*), 1,2-dimethylimidazole (*b*), and piperidine (*c*). Both *a* and *b* are in benzene whereas *c* is in neat piperidine. Excitation wavelength, 413.1 nm. The 607 cm^{-1} line is due to benzene.

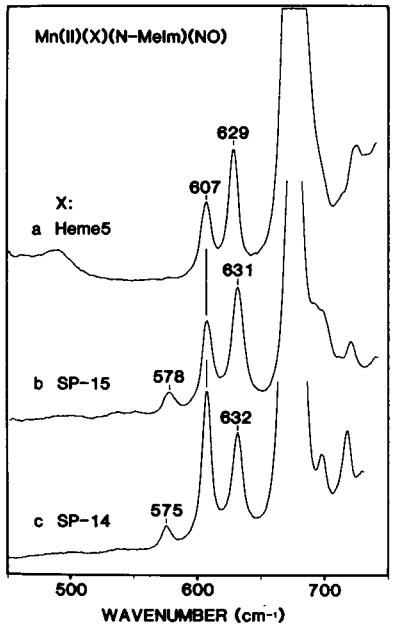

FIGURE 3. Low frequency (450–750 cm^{-1}) resonance Raman spectra of nitrosyl Mn heme-5, Mn SP-15, and Mn SP-14 complexes with *N*-methylimidazole as a base. Excitation wavelength, 413.1 nm.

hemes appears to be higher than that of the corresponding CTT IV and "open" heme complexes. The N—O stretching mode decreases with decreasing chain length (SP-15 > SP-14) and is much lower than that observed in the CTT IV complex. However, the N—O stretching of the manganese myoglobin complex is closer to the value found for the corresponding SP-14 complex. Replacement of *N*-methylimidazole by piperidine or 1,2-dimethylimidazole has only little effect on the frequency of the Mn—NO modes.

In Fig. 3 we compare low frequency (450–750 cm^{-1}) resonance Raman spectra of nitrosyl manganese heme-5, SP-15, and SP-14 complexes with *N*-methylimidazole as a *trans* ligand. It is apparent that the δ(Mn—N—O) mode (expected near 575 cm^{-1}) is not enhanced in the heme-5 complex, whereas this mode is readily seen at 578 cm^{-1} in the SP-15 complex and at 575 cm^{-1} in the SP-14 complex. Fig. 3 also shows the persistent increases in the Mn—NO stretching frequency (i.e., 629 → 631 → 532 cm^{-1}) as the steric hindrance increases from heme-5 to SP-15 and then to SP-14. In Fig. 4 we show that the N—O stretching frequency decreases (1,737 → 1,729 → 1,718 cm^{-1}) with increasing steric hindrance. Previously, Yu et al. (2) observed similar C—O stretching frequency decreases with increasing steric hindrance in carbonmonoxy Fe heme-5 (1,954 cm^{-1}), Fe SP-15 (1,945 cm^{-1}), Fe SP-14 (1,939 cm^{-1}), and Fe SP-13 (1,932 cm^{-1}).

In Table 1 we tabulate the observed δ(Mn—N—O), ν(Mn—NO), and ν(N—O) frequencies for heme-5, SP-15, SP-14, proto-IX dimethylester, proto-IX CTT IV, meso-IX CTT IV, and proto-IX Mb.

FORMATION OF Mn^{III}—NO COMPLEX IN MANGANESE INSECT HEMOGLOBIN CTT IV

In Fig. 5 we compare low frequency (150–750 cm^{-1}) resonance Raman spectra of Mn^{III} (meso-IX) CTT IV (*a*), Mn^{III} (meso-IX) CTT IV + NO (*b*), deoxy Mn^{II} (meso-IX) CTT IV (*c*), and nitrosyl Mn^{II} CTT IV (*d*) at pH 9.4. The corresponding high frequency (1,250–1,750 cm^{-1}) resonance Raman spectra are compared in Fig. 6. The spectra *b* in both Figs. 5 and 6 were obtained ~10 min after the addition of the nitric oxide (NO) gas to the

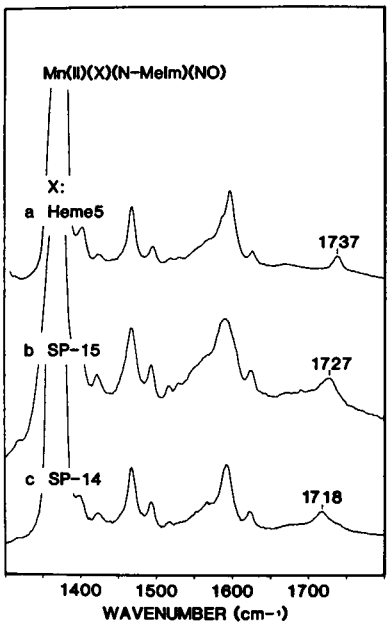

FIGURE 4 High frequency (1,300–1,800 cm^{-1}) resonance Raman spectra of nitrosyl Mn heme-5, Mn SP-15, and Mn Sp-14 complexes with *N*-methylimidazole as a base. Excitation wavelength, 413.1 cm.

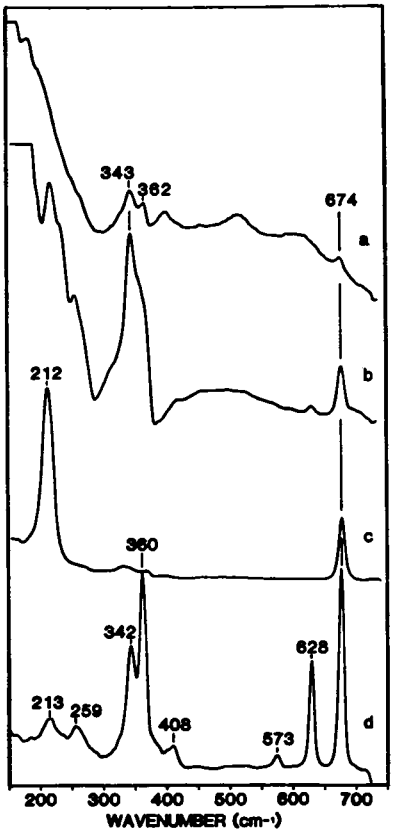

FIGURE 5 Low frequency (150–750 cm^{-1}) resonance Raman spectra of Mn^{III} (meso-IX) CTT IV (*a*), Mn^{III} (meso-IX) CTT IV + NO (~10 min after mixing) (*b*), deoxy Mn^{II} (meso-IX) CTT IV (*c*), and nitrosyl Mn^{II} (meso-IX) CTT IV (*d*). Excitation wavelength, 413.1 nm.

Raman cell containing the met Mn^{III} (meso-IX) CTT IV solution. On standing for ~6 h, the spectra *b* become identical to spectra *d*, which were obtained by adding NO gas directly to the deoxy Mn^{II} (meso-IX) CTT IV solution (i.e., after reduction of met Mn^{III} [meso-IX] CTT IV with dithionite under anaerobic conditions). Similar to the nitric oxide binding to ferric hemoproteins (11), the Mn^{III}—NO complex exhibits a tendency of spontaneous autoreduction to form the Mn^{II}—NO species. The NO molecule is able to donate one electron to the Mn^{III} center to form an intermediate Mn^{II}—NO^+, which is then reduced to Mn^{II}—NO by acquiring one electron from its environment. The nitrosyl complex of ferric horseradish peroxidase is very stable (12), presumably because of its unusually strong H-bonding between distal histidine and bound NO. The nitrosyl Mn^{III} (meso-IX) CTT IV complex is unstable, but we were still able to obtain its resonance Raman spectra with only small contributions from reduced Mn^{II}—NO species (e.g., Raman lines at 628 and 1,464 cm^{-1}, curves *b* in Figs. 5 and 6). The unligated Mn^{II} (meso-IX) CTT IV is characterized by the strong 212 cm^{-1} line (Fig. 5, curve *c*) which corresponds to the 215 cm^{-1} line of deoxy Mn^{II} Mb, first assigned by Yu and Tsubaki (13) to the Mn^{II}—N_ϵ (proximal histidine) stretching mode. This assignment has recently been confirmed by Parthasarathi and Spiro (14). The residual signal at 213 cm^{-1} in spectrum *d* of Fig. 5 may be due to photolyzed deoxy species.

Photolysis of Mn^{II}—NO complexes

All of the Mn^{II}NO complexes we have examined are photolabile and readily photolyzed by laser power >10 mW. Resonance Raman spectra of Mn^{II} (heme-5) (N-MeIm) (NO) obtained at 5, 25, 50, 100, and 200 mW are shown in Fig. 7. The so-called "oxidation-state marker" lines at 1,360 (deoxy) and 1,376 cm^{-1} (nitrosylated) change the relative intensities dramatically as the laser power increases from 5 to 200 mW. The ν(N—O) stretching at 1,736 cm^{-1} decreases and the ring mode at 1,466 cm^{-1} increases in intensity with increasing laser power.

DISCUSSION

Mn—N—O bending mode enhancement via ligand distortion

The enhancement of the Fe—C—O bending mode via ligand distortion was first demonstrated by Yu et al. (2) in their resonance Raman studies of carbonmonoxy

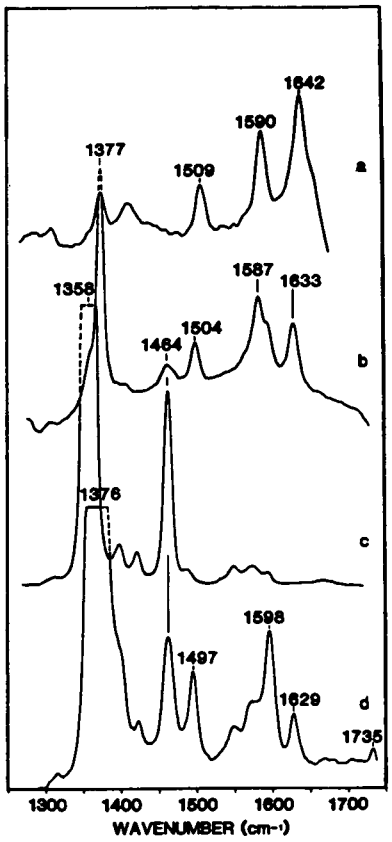

FIGURE 6. High frequency (1,250–1,750 cm^{-1}) resonance Raman spectra of Mn^{III} (meso-IX) CTT IV (*a*), Mn^{III} (meso-IX) CTT IV + NO (~10 min after mixing) (*b*), deoxy Mn^{II} (meso-IX) CTT IV (*c*), and nitrosyl Mn^{II} (meso-IX) CTT IV (*d*). Excitation wavelength, 413.1 nm.

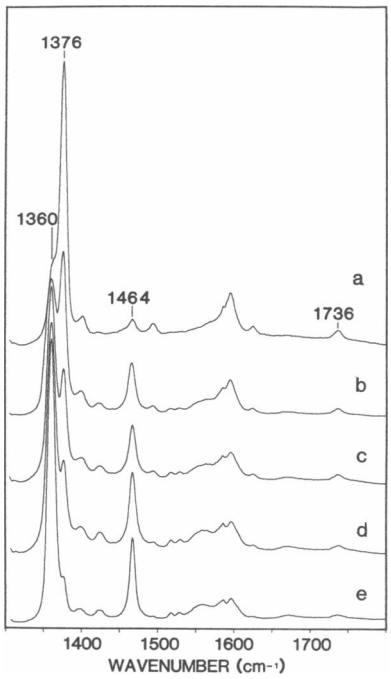

FIGURE 7. Photodissociation of nitric oxide by laser power: (*a*) 5 mW, (*b*) 25 mW, (*c*) 50 mW, (*d*) 100 mW, and (*e*) 200 mW. Excitation wavelength, 406.7 nm.

"strapped" hemes. In "open" heme complexes, where CO has a linear and perpendicular geometry, the Fe—C—O bending mode was not observed. However, when the Fe—C—O moiety is distorted by the molecular strap across one face of the heme, the Fe—C—O bending mode is readily detectable with Soret excitation. Fig. 3 shows that the enhancement of the Mn—N—O bending mode via ligand distortion is also possible in nitrosyl manganese "strapped" hemes (SP-15 and SP-14) with *N*-methylimidazole as a base. As shown in Fig. 3, the spectrum of nitrosyl manganese heme-5 ("unstrapped") exhibits no discernible Mn—N—O bending mode, expected near 575 cm^{-1}. In contrast, the Mn—N—O bending mode is detected at 578 and 575 cm^{-1} in nitrosyl manganese SP-15 and SP-14, in which the hydrocarbon straps cause the off-axis (tilted) bonding. Such a ligand distortion not only lowers the molecular symmetry but also increases the overlap between NO(π^*) and porphyrin (π^*) orbitals. Thus, upon Soret ($\pi - \pi^*$) excitation, the electron density can be transferred from porphyrin (π^*) to NO(π^*), causing a geometric distortion of the Mn—N—O moietry in the excited state from a linear to a bent structure (Jahn-Teller effect) (15). Hence, there exist two physical mechanisms (symmetry lowering and Jahn-Teller effect) cooperatively enhancing the resonance Raman intensity of the Mn—N—O bending mode.

The replacement of *N*-methylimidazole by 1,2-dimethylimidazole causes the tilting of the Mn—N_ϵ bond. Apparently, such a ligand distortion is also effective in causing the enhancement of the Mn—N—O bending mode. Resonance Raman spectra of nitrosyl manganese heme-5 complexes with *N*-methylimidazole and 1,2-dimethylimidazole are compared in Fig. 2 *A* (curves *a* and *b*). Similar comparison for nitrosyl manganese proto-IX dimethylester complexes is shown in Fig. 2 *B* (curves *a* and *b*). These two "unstrapped" heme systems do not have the Mn—N—O distortion. Thus, the Mn—N—O bending mode enhancement is apparently caused by the Mn—N_ϵ bond tilting in the 1,2-dimethylimidazole complexes. The symmetry lowering and Jahn-Teller effect may also be operative in the observed enhancement. An usually strong π backbonding in the Mn—N—O moiety (3) may be required. Previously, Yu et al. (2) in the studies of carbonmonoxy hemes did *not* observe the enhancement of Fe—C—O bending mode caused by the tilting of the Fe—N_ϵ bond in the 1,2-dimethyl-imidazole complexes.

The replacement of *N*-methylimidazole by piperidine also causes the enhancement of the Mn—N—O bending mode (see Fig. 2, *A* [*c*] and *B* [*c*]). This may be related to the distortion resulting from the steric interaction between H-atom and the atoms of the porphinato core. In fact, the x-ray crystallographic structures of Mn^{II} (tetraphenylporphyrin) (4-methylpiperidine) (NO) complex (16) indicates a long Mn—N_b bond (2.206 Å) to 4-methylpiperidine, caused by the steric interaction.

Effect of steric hindrance on the Mn^{II}—NO stretching mode

The $\nu(Mn^{II}—NO)$ frequency increases from 629 (heme-5) to 631 cm^{-1} (SP-15), and then to 632 cm^{-1} (SP-14) as the steric hindrance increases (see Fig. 3). Similar results have also been observed in carbonmonoxy "strapped hemes." The $\nu(Fe^{II}—CO)$ frequency increases 495 (heme-5) → 509 (SP-15) → 512 (SP-14) → 514 cm^{-1} (SP-13) (2). However, an inverse relation was found in cyanomet "strapped hemes." The $\nu(Fe^{III}—CN)$ frequency decreases with increasing steric hindrance: 451 (heme-5) → 447 (SP-15) → 447 (SP-14) → 445 cm^{-1} (SP-13) (17).

Because there is a predominant π backbonding in Mn^{II}—NO and Fe^{II}—CO, but not in $Fe^{III}—CN^-$, it appears that the increased interaction between the ligand (π^*) and the pyrrole *N*-atoms in the strapped hemes plays an important role in the increase of metal-ligand stretching frequency. It is of interest to note that the amount of the ν(Mn—NO) frequency increase with the steric hindrance is much smaller than that of the $\nu(Fe^{II}—CO)$ increase. This may be related to the fact that the Mn^{II}—NO bond is much stronger and hence more difficult to be tilted by the molecular strap. From heme-5 to SP-15, the $\nu(Mn^{II}—NO)$ increases only 2 cm^{-1}, whereas the $\nu(Fe^{II}—CO)$ increases 14 cm^{-1}. In these two systems, the degrees of the steric hindrance should be similar, because the M—X—O bond lengths are quite the same: 2.890 Å for the Fe—C—O bond in Fe^{II}(TPP) (pyridine) CO (18) and 2.920 Å for the Mn—N—O bond in Mn^{II}(TPP) (4-methylpiperidine) (NO) (16).

Effect of steric hindrance on the N—O stretching mode

In contrast with the $\nu(Mn^{II}—NO)$ mode, the ν(N—O) stretching frequency decreases with increasing steric hindrance: 1,736 (heme-5) → 1,727 (SP-15) → 1,718 cm^{-1} (SP-14), as shown in Fig. 4. In carbonmonoxy Fe^{II} "strapped hemes," the ν(C—O) frequency also decreases with increasing steric hindrance: 1,954 (heme-5) → 1,945 (SP-15) → 1,939 (SP-14) → 1,932 cm^{-1} (SP-13). This may be understood on the basis of an increase in π backbonding as the NO (or CO) ligand becomes distorted by the molecular straps. The increase in π electron density of the M—X—O moiety strengthens the M—X bond but weakens the X—O bond order because of the increasing density in the antibonding π^* orbital of the XO ligand.

Effect of various *trans* ligands on the Mn^{II}—NO stretching mode

Unlike carbonmonoxy Fe heme complexes, the *trans* ligand does not influence the strong Mn^{II}—NO bond. The $\nu(Mn^{II}—NO)$ frequency remains essentially the same (629 ± 1 cm^{-1}) when *N*-methylimidazole is replaced by 1,2-dimethylimidazole or piperidine (see Fig. 2 and Table 1). Previously, it was found that the stronger the *trans* ligand, the weaker the Fe^{II}—CO bond (2). However, the relation of this *trans* effect is reversed in cyanomet Fe^{III} heme systems, i.e., the $\nu(Fe^{III}—CN)$ frequency increases the increasing donor strength of the *trans* ligand (17). The origin of these effects might be due to different extents of the $d_\pi(M) \rightarrow \pi^*$ (XO) backbonding.

Interpretation of resonance Raman spectra of nitrosyl manganese CTT IV and myoglobin: N—O stretching mode as indicator of nitrosyl distortion and/or hydrogen bonding

The nitrosyl manganese CTT IV complexes (both proto-IX and meso-IX) exhibit ν(Mn—NO) at 628 cm^{-1} and ν(N—O) at 1,736 cm^{-1}, which are the same as those observed in the Mn^{II} (heme-5) (*N*-methylimidazole) (NO) complex (see Table 1). This is interpreted as the indication of a linear and perpendicular Mn—N—O moiety in the CTT IV complexes. The enhancement of the Mn—N—O bending mode at 574 cm^{-1} is then due to distortion of the proximal histidine-metal bond. In CTT IV there is no distal histidine and the bound NO is in a hydrophobic environment. The strong π back-bonding in the nitrosyl manganese CTT IV makes the tilting difficult and therefore unlikely. The nontilting Mn—N—O moiety is also true for nitrosyl manganese Mb on the basis of similarities of δ(Mn—N—O) and ν(Mn—NO). However, because of the distal histidine interaction with nitrosyl in Mb, the ν(N—O) frequency decreases by ~22 cm^{-1}, from 1,735 cm^{-1} observed in CTT IV. Therefore, the low frequency shifts of ν(N—O) in Mb and SP-14 are caused by two different mechanisms: distal histidine-NO interaction and ligand distortion, respectively.

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REFERENCES

1. Ward, B., C.-B. Wang, and C. K. Chang. 1981. Nonbonding steric effect on CO and O_2 binding to hemes. Kinetics of ligand binding in iron-copper cofacial diporphyrins and strapped hemes. *J. Am. Chem. Soc.* 103:5236–5238.
2. Yu, N.-T., E. A. Kerr, B. Ward, and C. K. Chang. 1983. Resonance Raman detection of Fe—CO stretching and Fe—C—O bending vibrations in sterically hindered carbonmonoxy strapped hemes. A structural probe of Fe—C—O distortion. *Biochemistry.* 22:4534–4540.
3. Lin, S.-H., N.-T. Yu, and K. Gersonde. 1988. Resonance Raman evidence for an unusually strong exogenous ligand-metal bond in a monomeric nitrosyl manganese hemoglobin. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 229:367–371.
4. Parthasarathi, N., and T. G. Spiro. 1987. Bonding in nitrosyl adducts of manganese-substituted hemoglobin and myoglobin monitored by resonance Raman spectroscopy. *Inorg. Chem.* 26:2280–2282.
5. Gersonde, K., H. Sick, A. Wollmer, and G. Buse. 1972. Heterotropic allosterism of monomeric hemoglobins of *Chironomus thummi thummi. Eur. J. Biochem.* 25:181–189.
6. LeMar, G. N., R. R. Anderson, V. P. Chacko, and K. Gersonde. 1983. High-resolution proton MNR as indicator of a silent mutation in the heme cavity of a monomeric allosteric hemoglobin. *Eur. J. Biochem.* 136:161–166.
7. Fuhrhop, J. H., and K. M. Smith. 1975. Porphyrins and Metalloporphyrins. K. M. Smith, editor. Elsevier Scientific Publishing Co., Amsterdam. 797.
8. Gersonde, K., H. Twilfer, and M. Overkamp. 1982. Bohr-effect and pH-dependence of electron spin resonance spectra of a cobalt-substituted monomeric insect hemoglobin. *Biophys. Struct. Mech.* 8:189–211.
9. Overkamp, M., H. Twilfer, and K. Gersonde. 1976. Conformation-controlled trans-effect of the proximal histidine in hemoglobins. An electron spin resonance study of monomeric nitrosyl-^{57}Fe-hemoglobins. *Z. Naturforsch.* 31C:524–533.
10. Yu, N.-T., and R. B. Srivastava. 1980. Resonance Raman spectroscopy of heme proteins with intensified vidicon detectors: studies of low frequency modes and excitation profiles in cytochrome c and hemoglobin. *J. Raman Spectrosc.* 9:166–171.
11. Benko, B., and N.-T. Yu. 1983. Resonance Raman studies of nitric oxide binding to ferric and ferrous hemoproteins: detection of Fe(III)—NO stretching, Fe(III)—N—O bending, and Fe(II)—N—O bending vibrations. *Proc. Natl. Acad. Sci. USA.* 80:7042–7046.
12. Yonetani, T., H. Yamamoto, J. E. Erman, Jr., J. S. Leigh, and G. H. Reed. 1972. Electromagnetic properties of hemoglobins. V. Optical and electron paramagnetic resonance characteristics of nitric oxide derivatives of metalloporphyrin-apohemoprotein complexes. *J. Biol. Chem.* 247:2447–2455.
13. Yu, N.-T., and M. Tsubaki. 1980. Resonance Raman spectra of manganese myoglobin and its azide complex. Assignment of a new charge-transfer band to azide (π) → porphyrin (π^*) transition. *Biochemistry.* 19:4647–4653.
14. Parthasarathi, N., and T. G. Spiro. 1987. Axial ligation of manganese (III/II) heme complexes and manganese-substituted myoglobin and hemoglobin from resonance Raman spectroscopy. *Inorg. Chem.* 26:3792–3896.
15. Yu, N.-T. 1986. Resonance Raman studies of ligand binding. *Methods Enzymol.* 130:351–409.
16. Piciulo, P. L., G. Rupprecht, and W. R. Scheidt. 1974. Stereochemistry of nitrosylmetalloporphyrins. Nitrosyl tetraphenylporphinato(1-methylimidazole) iron and nitrosyl tetraphenylporphinato(4-methylpiperidine) manganese. *J. Am. Chem. Soc.* 96:5293–5295.
17. Tanaka, T., N.-T. Yu, and C. K. Chang. 1987. Resonance Raman studies of sterically hindered cyanomet "strapped" hemes. Effects of ligand distortion and base tension on Iron-Carbon bond. *Biophys. J.* 52:801–805.
18. Peng, S. M., and J. A. Ibers. 1976. Stereochemistry of carbonylmetalloporphyrins. The structure of (pyridine) (carbonyl) (5, 10, 15, 20-tetraphenylporphinato) iron(II). *J. Am. Chem. Soc.* 98:8032–8036.