Title: LASER-EXCITED RAMAN SPECTROSCOPY OF BIOPOLYMERS

PROJECT ADMINISTRATION DATA

OCA contact: Kathleen R. Ehlinger 894-4820

Sponsor technical contact

DR. MICHELLE BROIDO
(301)496-7463

NAT INST OF GEN MEDICAL SCIENCES
NATIONAL INSTITUTES OF HEALTH
9000 ROCKVILLE PIKE
BETHESDA, MD 20892

Security class (U,C,S,TS) : U
Defense priority rating : N/A
Equipment title vests with: Sponsor

Administrative comments - ISSUED TO EXTEND TERMINATION DATE FROM 8/31/92 TO 8/31/93.
GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

Closeout Notice Date 11/02/94

Project No. G-33-G16__________

Center No. 10/24-6-Q5169-6A0_

Project Director YU N-T__________

School/Lab CHEMISTRY____

Sponsor DHHS/PHS/NIH/NATL INSTITUTES OF HEALTH_____________________

Contract/Grant No. 5 R01 GM18894-21__________

Contract Entity GIT_

Prime Contract No. __________________

Title LASER-EXCITED RAMAN SPECTROSCOPY OF BIOPOLYMERS____________________

Effective Completion Date 930831 (Performance) 931130 (Reports)

Closeout Actions Required: Y/N Date Submitted

Final Invoice or Copy of Final Invoice Y ___

Final Report of Inventions and/or Subcontracts Y ___

Government Property Inventory & Related Certificate N ___

Classified Material Certificate N ___

Release and Assignment N ___

Other ______________________________ N ___

Comments ____________________________________________________________________

**NOTE*** USE DHHS FORM FOR PATENT.

Subproject Under Main Project No. __________

Continues Project No. G-33-G15_________

Distribution Required:

Project Director Y

Administrative Network Representative Y

GTRI Accounting/Grants and Contracts Y

Procurement/Supply Services Y

Research Property Management Y

Research Security Services N

Reports Coordinator (OCA) Y

GTRC Y

Project File Y

Other ______________________________ N

NOTE: Final Patent Questionnaire sent to PDPI.
**NOTICE OF GRANT AWARD**

**DEPARTMENT OF HEALTH AND HUMAN SERVICES**

**NOTICE OF GRANT AWARD**

**ISSUED:** 06/08/92

**PUBLIC HEALTH SERVICE**

**RESEARCH**

**AUTHORIZED BY:** 42 USC 241 42 CFR 52

**WARDED BY:** NATIONAL INSTITUTE OF GENERAL MEDICAL SCIENCES

**TYPE OF AWARD:** RESEARCH

**TOTAL PROJECT PERIOD:** From 09/01/78 Through 08/31/93

**GRANT NUMBER:** R01 GM18894-21

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**Laser-Excited Raman Spectroscopy of Biopolymers**

**Principal Investigator/Program Director/Awardee:**

Yu, Nai-Teng  
PhD  

**Grantee Organization:**  
Georgia Inst. of Technology  
225 North Avenue, N W  
Atlanta, GA 30332

**Approved Budget**

<table>
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**Award Computation**

**Base Dollars x Rate Percentage = Indirect Costs $**

**Amount of This Award:** $186,723

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**Support Recommended for Remainder of Project Period:**

- **Budget Period:** 09/01/91 Through 08/31/93
- **Base Dollars x Rate Percentage = Indirect Costs $**

---

**THIS GRANT IS INCLUDED UNDER EXPANDED AUTHORITIES (PHS GRANTS POLICY STATEMENT REVISED 10/01/90)**

**PROGRAM ADMINISTRATOR:** Dr. Michelle Broido 301-496-7463

**GRANTS MANAGEMENT:** Lucy Clarke 301/496-7275

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**MARKS**

**Subject to availability of funds and satisfactory progress.**

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**ATTACHED FOR TERMS OF ACCEPTANCE AND ANY ADDITIONAL TERMS AND CONDITIONS.**

---

**COMMON ACCOUNTING NUMBER:** 88243576

**CRS/ENTITY IDENTIFICATION NO.:** 1586002023A1

**PHS LIST NO./OBJECT CLASS CODE:** /41.4E

**DOCUMENT NUMBER:** (08)R1GM18894E

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**PROGRAM OFFICIAL:**

- **PHS Grants Management Official:** Carol L. Tippery

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**PROGRAM ACTIVITIES OFFICE ASSOC. DIRECTOR PROGRAM ACTIVITIES, NIGMS**

---

**Sue Shaffer, PhD**

**Associate Director for Program Activities**


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**CAS/Entity Identification No.:** 1586002023A1

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**MS 1533**

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TERMS AND CONDITIONS

Terms of acceptance: This grant is subject to the terms and conditions incorporated either directly or by reference in the following: (1) The grant program legislation cited on the first page; (2) The grant program regulations cited on the first page; (3) This award notice including terms and conditions, if any, noted below or attached to this notice; (4) PHS Grants Policy Statement including addenda in effect as of the beginning date of the budget period; (5) 45 CFR Part 74 or 92 as applicable. In the event there are conflicting or otherwise inconsistent policies applicable to the grant, the above order of precedence shall prevail. Acceptance of the grant terms and conditions is acknowledged by the grantee when funds are drawn or otherwise obtained from the grant payment system.

1. This award is revised to extend the budget/project period for 12 months in accordance with the grantee’s notification letter dated 5/29/92.

2. Any general program income accruing under this grant may be used in accordance with the additional costs alternative described in 45 CFR Part 74 Subpart F [74.42(e)].

3. This award includes $15,204 for the purchase of lasser tubes. These funds are restricted solely for that purpose and may not be rebudgeted.

4. This project is in its final year of recommended support. In the event the project is not renewed or further extended, Public Health Service policy requires submission of the following final reports within 90 days after the grant’s final budget period expires:

   (1) Invention Statement (HHS-568)
   (2) Financial Status Report (SF-269)
   (3) Progress Report

Failure to submit these required reports, when due, may result in the imposition of special award provision or the withholding of support for other eligible projects or activities involving the grantee organization or the individual responsible for the delinquency.
May 26, 1992

CONTRACTING OFFICER
CRB RM 246
GEORGIA INST. OF TECHNOLOGY
ATLANTA, GA 30332

May 1992 Council
Re: 2R01GM18894-22 4260MB

Dear Mr. Business Official:

The application referenced above was submitted by your institution for review and consideration for funding. The initial review group ranked this application in the lowest third of the applications it reviewed. Consequently, in accordance with our new review procedures, the application was not reviewed by the National Advisory General Medical Sciences Council and cannot receive an award. We will therefore withdraw it administratively. Please be assured that these actions will in no way affect any application that the principal investigator may wish to submit in the future.

If you have any questions, please feel free to contact the program administrator whose name and telephone number are given below.

Sincerely Yours,

W. Sue Shafer, Ph.D.
Associate Director for
Program Activities
National Institute of
General Medical Sciences

The Program Administrator for your application is:
Michelle S. Broido, Ph.D.
(301) 496-7463

cc: Nai-Teng Yu, Ph.D.
Title of the Project: Laser-excited Raman Spectroscopy of Biopolymers


Institution: Georgia Institute of Technology

The main objectives of the project during the above period are to explore, develop and exploit the information content of resonance Raman spectra of hemoproteins with special emphasis on the detection / interpretation of metal-axial ligand vibrations and their utilization for understanding the mechanisms of protein control of heme reactivity. We have collaborated extensively with Professor Klaus Gersonde of Technical University of Aachen (Germany), and Dr. K. Nagai of Medical Research Council (UK). Prof. Gersonde had provided us with the samples of insect hemoglobin Chironomus thummi thummi, while Dr. Nagai supplied us with human mutant Hb's obtained via site-directed mutagenesis. These collaborations have been quite fruitful, producing numerous publications as listed below. We have also carried out resonance Raman studies on ligand binding to model compounds, which provide new insight into the exact nature of metal-ligand bonds and their relations with ligand binding affinities. In addition, we have employed near-infrared Fourier Transform Raman technique to study fluorescent or photolabile biomolecules, which were not possible for Raman studies using excitation in the visible region.

The personnel who have worked on the project:

Yu, Nai-Teng          P.I.
Gersonde, K.          Research Collaborator (Germany)
Nagai, K.             Research Collaborator (UK)
Lin, S. H.            Graduate Student, completed his Ph.D. degree
Lee, B.-S.            Postdoctoral Research Associate
Lipscomb, L.          Graduate Student, completed her Ph.D. degree
Nie, S.               Postdoctoral Research Associate, becoming Assistant Professor at Indiana University
Tsubaki, M.           Research Collaborator, Summer Res. Associate
Castillo, C.          Graduate Student, completed her Ph.D. degree
Zhang, Fuli           Research Associate
Liu, H.-H.            Graduate Student, completed her M.Sc. degree
Renaud, J.-P.         Vist. Scientist (collaborator) (France)
Tame, Jeremy          Vist. Scientist (collaborator) (UK)
Cai, Ming-Zhi         Research Associate
Chopra, Manu          Graduate Student, will complete his Ph.D. in March 1995
Feng, Sibo            Graduate Student, completed M.Sc. degree; now a Ph.D. candidate at Harvard Univ.
Li, Xiao-Yuan         Research Collaborator (Hong Kong)

Publications resulting from this funded project:


Significant findings may be summarized as follows:

(a) We made the first comparison of the \(v(\text{Fe}^{II-}\text{NO})\) stretching frequencies in iron porphyrins (\(-527\ cm^{-1}\)) and hemoproteins (\(-554\ cm^{-1}\)) to assess the protein's effect on the \(\text{Fe}-\text{NO}\) bond. The electronic trans effect was investigated by preparing complexes with N-methylimidazole, pyridine, and tetrahydrofuran as proximal ligands, and steric effects were assessed by comparing hindered (1,2-dimethylimidazole) and unhindered (N-methylimidazole) axial bases. Surprisingly, the \(\text{Fe}^{II-}\text{NO}\) bond strength proved insensitive to alterations in the trans base. The results were contrasted with \(\text{Fe}^{II-}\text{CO}, \text{Fe}^{II-}\text{O}_2,\) and \(\text{Fe}^{II-}\text{CN}\) studies, in which the iron-ligand bond strength was found to be dramatically affected by properties of the proximal ligand.

(b) The nitridochromium (V) porphyrin complex was produced by UV irradiation of the azide complexes of the chromium (III)-protoporphyrin-IX. The Cr(V)-N stretching mode was observed at 1010 cm\(^{-1}\) by resonance Raman spectroscopy. The force constant of 6.7 mdyne/\(\AA\) for the Cr(V)-N bond was found, consistent with that for the metal-nitrogen triple bond.

(c) Surface-Enhanced hyper-Raman Spectroscopy: We have demonstrated the effect of excitation pulse width on hyper-Raman intensity. We have shown that SEHR technique provides new vibrational information for non-centrosymmetric molecules. Surface-enhanced hyper-Raman signals from gold and copper colloids were demonstrated.

(d) Raman Evidence for Non-coupling of individual 2- and 4-Vinyl Vibrational Modes in Cyano-met Hb: By studying cyano-met CTT Hbs reconstituted with protoheme-IX selectively deuterated at vinyl and 2,4-vinyls, pemptoheem, isopemtoheem, and symmetric hemes (protoheme-III and -XIII), we have obtained unequivocal evidence that the highly localized vinyl \(C=C\) stretching vibrations at the 2 and 4 positions are non-coupling and inequivalent.

(e) The Origin of the Distal Steric Effect in Carbonmonoxy Hemoglobin: We have obtained new insight into the origin of the distal effect in Hb\(\cdot\text{CO}\). Dr. Nagai provided us with human mutant Hbs having: (1) His(\(\alpha\)E7) replaced by Gln or Gly; (2) His(\(\beta\)E7) by Gln, Val, Gly or Phe; (3) Val(\(\alpha\)E11) by Ala, Leu or Ile; (4) Val(\(\beta\)E11) by Ala, Met, Leu or Ile; and (5) Phe(CD1) by Gly or Tyr. The effects of
these mutations on the vibrational properties of the Fe-C and C-O bonds in carbonmonoxy Hb A have been studied by Soret-excited resonance Raman spectroscopy. It is concluded that the origin of the distal steric effect that causes the off-axis CO bonding is not the steric bulk of the E-7 residue. Instead, it may be the repulsive polar interactions between the lone-pair electrons of the \( N_e \) and those of the carbonyl oxygen.

(f) **Resonance Raman Probe of Heme-Rotational Dosorder in Cyano-met CTI Hbs:** We have identified two Fe-C-N Bending Vibrations attributable to heme-rotational components in deutero-IX and meso-IX reconstituted CTIs.

(g) **Near-Infrared FT-Raman Spectroscopy for the Studies of Photolabile and Fluorescent Biomolecules:** For the first time, we reported the detection of the stretching vibration of the Co-C bond in photolabile B\(_{12}\) and model compounds by near-IR excited FT-Raman spectroscopy. This opens up the possibility of directly monitoring the Co-C bond in various B\(_{12}\) complexes.

(h) **Assessment of Factors Affecting the Co-C Bonds in B\(_{12}\) Models:** We have assessed the importance of various factors such as trans electronic effect, trans steric effect, environmental effect, which influence the Co-C bond stretch (hence Co-C bond strength).

(i) **No Change in Nitrosyl Bonding Geometry in Mb-NO between 20 °C and 77 °K:** By single crystal EPR spectroscopy, the Fe-N-O bond angle in Mb.NO was found to change by temperature from 153° (at 20 °C) to 109° (at 77 °K). Unexpectedly, we found no change in the Fe-NO stretch at 554 cm\(^{-1}\) between the two temperatures, indicating no change in nitrosyl bonding geometry. The discrepancy simply indicates that EPR data need to be re-interpreted.

(j) **Unusual Ligand Binding to Liver Fluke Dd Hemoglobin:** We have detected the Fe(II)-NO, Fe(II)-CO, and Fe(II)-O\(_2\) stretching vibrations of Dd Hb at 567, 571 and 478 cm\(^{-1}\), respectively. Compared with the Fe(II)-NO stretching mode of Mb.NO and Hb.NO at 554 and 551 cm\(^{-1}\), the \( v(Fe^{II}-NO) \) frequency for Dd Hb.NO is ~15 cm\(^{-1}\) higher. On the other hand, the Fe\(^{II}\)-CO stretch of Dd Hb.CO is ~32 cm\(^{-1}\) lower than that of Mb-CO (507 cm\(^{-1}\)) and HbA-CO (512 cm\(^{-1}\)). The replacement of distal His by Tyr in Dd Hb indeed has a profound effect on the \( v(Fe^{II-NO}) \) and \( v(Fe^{II-CO}) \) vibrations.
Resonance Raman Investigation of Nitric Oxide Bonding in Iron Porphyrins: Detection of the Fe–NO Stretching Vibration

Leigh Ann Lipscomb,¹ Bao-Shiang Lee,¹ and Nai-Teng Yu²,3,4

School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, Georgia 30332, and Department of Chemistry, The Hong Kong University of Science and Technology, Kowloon, HK

Received September 20, 1991

With Soret-excited resonance Raman spectroscopy (RRS), we have detected the ω(FeI–NO) and ω(FeII–NO) stretching vibrations for several iron porphyrins, including octaethylporphyrin (OEP), tetraporphyrin (TPP), heme-5, SP-13, SP-14, and SP-15. This result enables us to make the first comparison of the ω(FeI–NO) stretching frequencies in iron porphyrins (~527 cm⁻¹) and hemoproteins (~554 cm⁻¹), which is of interest if the protein's effect on the Fe–NO bond is to be assessed. Solvent effects on the FeI–NO bond strength were significant; for FeII(OEP)(pyridine)(NO), the ω(FeI–NO) stretching frequency was observed 5 cm⁻¹ higher in CH₂Cl₂ (~527 cm⁻¹) than in benzene (~522 cm⁻¹). The electronic trans effect was investigated by preparing complexes with N-methylimidazole, pyridine, and tetrahydrofuran as proximal ligands, and steric effects were assessed by comparing hindered (1,2-dimethylimidazole) and unhindered (N-methylimidazole) axial bases. Interestingly, the FeII–NO bond strength proved insensitive to alterations in the trans base. These results are contrasted with previous FeII–CO, FeIII–O₂, and FeII–CN studies, in which the iron–ligand bond strength was found to be dramatically affected by properties of the proximal ligand.

Introduction

Nitrosoyl complexes have been extensively investigated by X-ray diffraction,¹¹ infrared,¹² electronic spin resonance (ERS),¹³–¹⁰,¹¹–¹⁳ visible absorption,¹¹–¹³ extended X-ray absorption fine structure (EXAFS),¹⁴ and resonance Raman spectroscopy (RRS).¹⁵–²³ It is known that nitric oxide (NO) binds to both ferric and ferrous hemes with an unusually high affinity.²⁴ With RRS, the Fe–NO bond can be readily monitored in terms of its stretching mode.

The first detection of ω(FeII–NO) in hemoproteins (554 cm⁻¹) was reported in 1977 by Chottard and Mansuy.²⁵ Somewhat later, Tsubaki and Yu²⁶ used RRS to study ω(FeII–NO) (551 cm⁻¹) and ω(N–O) (~1623 cm⁻¹) in nitrosyl-hemoglobin (A (HbA) and myoglobin. For ferric hemoproteins, Benko and Yu²⁷ were the first to observe ω(FeII–NO) and ω(FeI–NO) in nitrosyl complexes of HbA and horseradish peroxidase. Recently, Choi et al.²⁸ have reported the resonance Raman detection of ω(FeII–NO) for the five-coordinate FeII(TPP)(NO). However, no infrared or resonance Raman detection of ω(FeII–NO) and ω(FeI–NO) has been reported for the six-coordinate FeII(porphyrin)(base)(NO) in the literature. Such information is essential for assessing the protein's influence on the Fe–NO moiety, since the protein systems are also six-coordinate (with histidine as the proximal base).

In this paper, we describe Soret-excited RRS studies of nitric oxide complexes of ferric and ferrous porphyrins. With nitric oxide substitution, the ω(FeII–NO) and ω(FeI–NO) stretching modes have been clearly identified at ~602 and ~524 cm⁻¹, respectively. The protein's influence on the ω(FeII–NO) stretching frequency (~30 cm⁻¹) is indeed significant. We have also investigated the effects of the solvent and trans base on the FeII–NO bond. Trans bases of different strengths (N-methylimidazole, pyridine, tetrahydrofuran) were studied, and sterically hindered (1,2-dimethylimidazole) and unhindered (N-methylimidazole) bases were also compared. We were surprised to find that the FeII–NO bond is insensitive to the nature of the trans ligand. Solvent effects on the ω(FeII–NO) stretching frequency were investigated by comparing complexes of FeII(OEP)(pyridine)(NO) in benzene, tetrahydrofuran, carbon tetrachloride, chloroform, and methylene chloride. The ω(FeII–NO) stretching frequency was generally observed to increase for solvents of higher dipole moment.

Experimental Section

Iron(III) octaethylporphyrin (OEP) chloride was obtained from Strem Chemicals (Newburyport, MA), while iron(III) meso-tetraphenylporphyrin (TPP) bromide was purchased from Midcentury (Posen, IL). Both porphyrins were used without further purification. Heme 5, as well as ferrous HbA–NO) was reported in 1977 by Chottard and Mansuy.²⁵ Somewhat later, Tsubaki and Yu²⁶ used RRS to study ω(FeII–NO) (551 cm⁻¹) and ω(N–O) (~1623 cm⁻¹) in nitrosyl-hemoglobin (A (HbA) and myoglobin. For ferric hemoproteins, Benko and Yu²⁷ were the first to observe ω(FeII–NO) and ω(FeI–NO) in nitrosyl complexes of HbA and horseradish peroxidase. Recently, Choi et al.²⁸ have reported the resonance Raman detection of ω(FeII–NO) for the five-coordinate FeII(TPP)(NO). However, no infrared or resonance Raman detection of ω(FeII–NO) and ω(FeI–NO) has been reported for the six-coordinate FeII(porphyrin)(base)(NO) in the literature. Such information is essential for assessing the protein's influence on the Fe–NO moiety, since the protein systems are also six-coordinate (with histidine as the proximal base).

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as the strapped hemes (FeSP-13, FeSP-14, FeSP-15), was synthesized according to literature methods. The NO complexes of these ferric porphyrins (200 μM) were prepared with tetrahydrofuran (THF, 0.10 M), N-methylimidazole (N-Melm, 0.15 M), 1,2-dimethylimidazole (1,2-DiMelm, 0.15 M), and pyridine (0.15 M) as proximal bases in CH₂Cl₂ solution. For preparation of the ferrous complex, methanol (0.1 M) was added to the porphyrin/CH₂Cl₂/base solution prior to the addition of the NO gas; methanol is known to act as a catalyst in the autoreduction of ferric porphyrins by NO. The sample solution was placed in a quartz Raman cell, which was immediately sealed and evacuated. Nitric oxide (from Aldrich, Milwaukee, WI) was then introduced at a pressure of ~1 atm. The isotope-substituted complexes were prepared similarly with ¹⁵N(NO) (Bio-Rad Laboratories, Richmond, CA), and ¹³N(NO) (ICON Services, Summit, NJ).

All reagents were purified prior to use. Methylene chloride was stirred over concentrated sulfuric acid, neutralized with a 10% NaHCO₃ solution, dried over anhydrous MgSO₄, and distilled from phosphorus pentoxide. Pyridine and N-Melm were distilled from KOH, while 1,2-DiMelm and methanol were distilled from Na and Mg, respectively. Reagent grade tetrahydrofuran was used as received from Fisher Scientific (Fair Lawn, NJ).

The multichannel laser Raman system has been described previously. The 406.7 nm line of a Spectra-Physics (Mountain View, CA) Model 171 krypton ion laser was employed for excitation. The laser used to obtain each spectrum is listed in the figure captions. To minimize photodissociation and localized heating, the Raman cell was kept spinning throughout the entire data acquisition process. The entrance slit of the monochromator had a width and height of 100 μm and 1 cm, respectively. Fenchone was used to calibrate the spectra; peak positions are considered accurate to ±2 cm⁻¹.

Results and Discussion

Identification of the Fe–NO Stretching Vibrations. We have studied the effects of nitric oxide isotope substitution on the low-

(100–700 cm⁻¹) and high (1300–1900 cm⁻¹) frequency regions of FeIII(OEP)(THF)(NO) spectra. (See Figure 1 for structures of all the iron porphyrins studied in this work.) The substitutions reveal one isotope-sensitive line at 527 cm⁻¹ for FeIII(OEP)(THF)–(¹⁵N(NO), which shifts to 519 cm⁻¹ (¹⁴N(NO) and to 512 cm⁻¹ (¹³N(NO) (see Figure 2). It is clear that this line corresponds to the 554-cm⁻¹ mode in nitrosyl HbA assigned by others as ω–(FeIII–NO). Careful examination of the spectral region from 400 to 500 cm⁻¹ reveals no ω(FeIII–N–O) bending mode for any of the porphyrins studied.

Because the autoreduction of FeIII(OEP)(pyridine)(NO) to FeII(OEP)(pyridine)(NO) is very slow (~48 h), we were able to study the effects of nitric oxide isotope substitution on the low-

(100–700 cm⁻¹) and high (1300–1900 cm⁻¹) frequency regions of FeIII(OEP)(THF)(NO) spectra. (See Figure 1 for structures of all the iron porphyrins studied in this work.) The substitutions reveal one isotope-sensitive line at 527 cm⁻¹ for FeIII(OEP)(THF)–(¹⁵N(NO), which shifts to 519 cm⁻¹ (¹⁴N(NO) and to 512 cm⁻¹ (¹³N(NO) (see Figure 2). It is clear that this line corresponds to the 554-cm⁻¹ mode in nitrosyl HbA assigned by others as ω–(FeIII–NO). Careful examination of the spectral region from 400 to 500 cm⁻¹ reveals no ω(FeIII–N–O) bending mode for any of the porphyrins studied.

Nitric Oxide Bonding in Iron Porphyrins


Figure 2. Nitric oxide isotope effects on the low-frequency region (420−650 cm⁻¹) spectra of Fe(II)(OEP)(THF)(NO). λₘ = 406.7 nm; laser power = 40 mW at the sample. Because only small portions of the spectra are displayed in each figure, it should be mentioned that all features of our RRS data are in good agreement with those previously published for metallooctaethylporphyrins.⁴⁰

(CO)³¹ is much longer than the Fe(III)-NO bond investigated in this paper, because the observed ϵ(Fe(III)-CO) stretch (486 cm⁻¹)³⁰ is much lower than ϵ(Fe(III)-NO) of 602 cm⁻¹ reported here. It is well-known³⁰ that the stretching frequency can be correlated with the force constant and bond length. We suggest that the Fe(III)-NO bond of Fe(III)(SP-14)(pyridine)(NO) and Fe(III)(SP-15)-(pyridine)(NO) (NO) is short enough to prevent geometric distortion of the Fe(III)-NO linkage by the strap. Therefore, Fe(III)-NO maintains its preferred linear configuration, and δ(Fe(III)-N-O) is not enhanced.

We have also identified ϵ(Fe(II)-NO) and ϵ(Fe(III)-NO) for several other iron porphyrins, and all of the results are given in Table I. It should be noted that unsuccessful attempts to observe ϵ(Fe(II)-NO) in porphyrins were previously reported by Strong et al.²⁰ Both the excitation wavelength (454.5 nm) and detector (photomultiplier tube) were different from those employed in this study.

Comparison of [Fe(III)-NO] with the Isoelectronic [Fe(II)-CO] System. In the absence of distal steric hindrance, both the Fe(III)-NO and Fe(II)-CO moieties are known to adopt linear configurations normal to the heme plane. X-ray and neutron scattering crystal structures of carbonmonoxy adducts of hemoproteins³³⁻⁴² reveal that nonbonded contacts in the heme pocket force the bound CO out of its preferred linear configuration. Li and Spiro⁴³ have suggested that these protein-induced distortions result mainly in tilting rather than bending of the Fe(II)-CO unit. Yu et al.,³⁰ in studies of sterically hindered carbonmonoxy strapped hemes, observed that distortions in the Fe(II)-CO linkage resulted in an increase in the ϵ(Fe(II)-CO) stretching frequency. It is of interest to ask whether or not similar distortions occur in NO adducts of ferric hemoproteins.

The similar υ(FeIII–NO)’s for ferric HRP–NO (604 cm⁻¹), ferric Mb–NO (595 cm⁻¹),23 and FeIII(OEP)(pyridine)(NO) (602 cm⁻¹) indicate that the protein’s effect on the FeIII–NO bond is small, compared with the FeII–CO system. Previously, the υ(FeII–CO) stretching frequency was observed 16 cm⁻¹ higher for MbCO (512 cm⁻¹)44 than for FeII(OEP)(N-methylimidazole)-CO (496 cm⁻¹).32 For Mb, the relative stretching frequencies of υ(FeIII–NO) (595 cm⁻¹) and υ(FeII–CO) (512 cm⁻¹)45 suggest that the FeIII–NO bond is stronger than the FeII–CO bond. However, to avoid being misled by mass effects, we calculated the υ(FeII–CO) stretching frequency (for Mb–CO) on the basis of corrected mass normalized to the FeIII–NO system. We then obtained a value of 501 cm⁻¹ for υ(FeII–CO). These frequency numbers (501 cm⁻¹ for FeII–CO, 595 cm⁻¹ for FeIII–NO) do indeed correlate with a force constant that is highest for the FeII–NO bond. While X-ray data for nitrosyl adducts of ferric hemoproteins are unavailable, a comparison of the stretching frequencies indicates that the FeIII–NO bond is strongest and hence the shortest. Evidently the strength of the FeIII–NO bond renders it less susceptible to protein distortions. It is plausible that this bond is strong enough not to sterically interfere with bulky amino acid residues near the binding site.

**Protein Effect.** The results presented here facilitate the first comparison of the υ(FeII–NO) stretching frequencies in model compounds and hemoproteins. For FeII(OEP)(N-Melm)(NO), υ(FeII–NO) was detected at 524 cm⁻¹, ca. 30 cm⁻¹ lower than that observed for nitrosyl HbA (∼554 cm⁻¹).15 An interesting observation evolves from the comparison of protein effects on FeII–NO and FeII–O2. While both of these moieties maintain bent geometries, the protein effect on FeII–O2 is much smaller than that on FeII–NO, and the NO binding to FeII–HEME in Hb is ~1000 times stronger than Õ2 binding.24

To obtain a more definitive assessment of the protein’s effect on the FeII–NO bond, we have carried out resonance Raman studies of FeII[PP](N-Melm)(NO) (PP = protoporphyrin IX) in aqueous solution at alkaline pH. Because iron protoporphyrin IX is present in Hb and Mb, comparisons of the υ(FeII–NO) stretching frequency in Mb–NO and FeII[PP](N-Melm)(NO) should reflect only the effects of the protein. Unfortunately, slight background fluorescence prevented observation of υ(FeII–NO) for the protoporphyrin IX system. But the comparisons we have made between FeII(OEP)(N-Melm)(NO) and Mb–NO and Hb–NO are still useful, because alterations in the heme group have only a slight effect on υ(FeII–NO) (see Table 1). Furthermore, the υ(FeII–CO) stretching frequencies reported for FeII[PP](N-Melm)(CO) (497 cm⁻¹)15 and FeII(OEP)(N-Melm–CO) (496 cm⁻¹)24 are very similar.

**Trans Effect.** Assuming that the FeII–N–O geometry is not affected by a change in the trans ligand, the υ(FeII–NO) stretching frequency may be correlated with the FeII–NO bond strength (or bond length). In this work, we have examined the influence of trans ligands on the υ(FeII–NO) stretching frequency. The ligands studied were N,N,N,N-tetramethyldiamine (N-Melm), pyridine, and tetrahydrofuran (THF). It is also interesting to compare the υ(FeII–NO) stretching frequency in complexes with N,N,N,N-tetramethyldiamine and 1,2-dimethylimidazole (1,2-DiMelm) as proximal bases. The methyl group in the 2-position of 1,2-Dimelm sterically interferes with the porphyrin plane, thereby lengthening the Fe–N bond (N, represents the N atom of the proximal base). Iron porphyrin complexes with 1,2-DiMelm provide an excellent model for the tense (or low affinity) state of hemoglobin.46 Results for FeII(OEP)(N-Melm)(NO), FeII(OEP)(1,2-DiMelm)(NO), and FeII(OEP)(pyridine)(NO) are shown in Figure 4, while the spectrum of FeII(OEP)(THF)(NO) is displayed in Figure 1. Interestingly, υ(FeII–NO) proved insensitive to both electronic and steric trans effects. Kincaid et al.24 have previously noted similar behavior of υ(FeII–NO) of nitrosyl adducts of ferrous cytochrome P450cam. The authors report that the υ(FeII–NO) stretching frequency was not altered by proximal cysteine ligation. Apparently, bending of the Fe–N–O linkage substantially increases the energy of the dπω→ω orbital.47,48 Thus rendering the transfer of a lone pair on the axial sulfur energetically unfavorable.

To make certain that we were not studying the bis(nitrosyl) adduct, FeII(OEP)(NO)2, we prepared this adduct and studied its Raman and absorption spectra. In the absence of any base, FeII(OEP)(NO)2 can be readily formed under our experimental conditions.49 The absorption spectrum obtained from this complex was significantly different from that obtained for the FeII(OEP)–(base)(NO) complexes (data not shown). Additionally, we observed an isotope-sensitive line at 519 cm⁻¹ in the resonance Raman spectrum of FeII(OEP)14(N=O)2; this line shifts to 514 cm⁻¹ upon substitution by 15N=O. The υ(FeII–NO) stretching frequency detected for FeII(OEP)(NO)2 (519 cm⁻¹) is indeed significantly different from that observed for FeII(OEP)(base)(NO) (~527 cm⁻¹).

Additionally, we have monitored the υ(FeII–NO) stretching frequency under conditions which we can be positive that the trans ligand is coordinated. FeII(OEP)(pyridine)(NO) was prepared in neat pyridine, and υ(FeII–NO) was detected at 526 cm⁻¹ (spectrum not shown). υ(FeII–NO) was also observed at

Table II. Comparison of the Trans Effects for CO, O₂, CN, and NO Complexes of Iron Porphyrins: Results from Resonance Raman Spectroscopy

<table>
<thead>
<tr>
<th>complex</th>
<th>ν(Fe⁻⁻⁻⁻⁴-CO) (cm⁻¹)</th>
<th>ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe⁷⁺(TpivPP)(THF)(CO)</td>
<td>527</td>
<td>32</td>
</tr>
<tr>
<td>Fe⁷⁺(TpivPP)(pyridine)(CO)</td>
<td>486</td>
<td>32</td>
</tr>
<tr>
<td>Fe⁷⁺(TpivPP)(N-Melnl)(CO)</td>
<td>489</td>
<td>32</td>
</tr>
<tr>
<td>Fe⁷⁺(TpivPP)(1,2-DiMelm)(CO)</td>
<td>496</td>
<td>32</td>
</tr>
</tbody>
</table>

Complex | ν(Fe⁻⁻⁻⁻⁴-O₂) (cm⁻¹) | ref |
<table>
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<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe⁷⁺(TpivPP)(N-Melnl)(O₂)</td>
<td>571</td>
<td>51</td>
</tr>
<tr>
<td>Fe⁷⁺(TpivPP)(1,2-DiMelm)(O₂)</td>
<td>561</td>
<td>51</td>
</tr>
</tbody>
</table>

Complex | ν(Fe⁻⁻⁻⁻⁴-CN) (cm⁻¹) | ref |
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Fe⁷⁺(SP-15)(N-Melnl)(CN)</td>
<td>447</td>
<td>52</td>
</tr>
<tr>
<td>Fe⁷⁺(SP-15)(pyridine)(CN)</td>
<td>456</td>
<td>52</td>
</tr>
<tr>
<td>Fe⁷⁺(SP-15)(1,2-DiMelm)(CN)</td>
<td>446</td>
<td>52</td>
</tr>
</tbody>
</table>

525 cm⁻¹ for both Fe⁷⁺(OEP)(N-methylimidazole)(NO) (in neat N-methylimidazole) and Fe⁷⁺(OEP)(1,2-dimethylimidazole)- (NO) (in neat 1,2-dimethylimidazole). Clearly, these results unambiguously demonstrate that ν(Fe⁻⁻⁻⁻⁴-CN) is insensitive to electronic/steric properties of the trans base.

This lack of an electronic trans effect has been noted previously for the Mn⁻⁻⁻⁻⁴-NO system. Yu et al. reported almost no difference in the ν(Mn⁻⁻⁻⁻⁴-NO) stretching frequencies observed for Mn⁷⁺ (heme-5)(N-Melm)(NO) (629 cm⁻¹) and Mn⁷⁺(heme-5)(pyridine)(NO) (630 cm⁻¹). In contrast, other ligand systems such as Fe⁻⁻⁻⁻⁴-CO, Fe⁻⁻⁻⁻⁴-O₂, and Fe⁻⁻⁻⁻⁴-CN are known to exhibit significant trans effects. In Table II, we compare the sensitivities of the Fe⁻⁻⁻⁻⁴-CO, Fe⁻⁻⁻⁻⁴-CN, and Fe⁻⁻⁻⁻⁴-NO bonds to alterations in the proximal base. Results in Table II indicate that, while the ν(Fe⁻⁻⁻⁻⁴-CO) stretching frequency for Fe⁻⁻⁻⁻⁴-(TpivPP)(pyridine)(CO) is 41 cm⁻¹ lower than that of Fe⁻⁻⁻⁻⁴-(TpivPP)(THF)(CO), the position of ν(Fe⁻⁻⁻⁻⁴-CN) is identical (527 cm⁻¹) for Fe⁻⁻⁻⁻⁴(OEP)(pyridine)(NO) and Fe⁻⁻⁻⁻⁴(OEP)(THF)(NO). These resonance Raman results are supported by X-ray crystallographic studies, and Table III compares the sensitivities of the Fe⁻⁻⁻⁻⁴-CO, Fe⁻⁻⁻⁻⁴-CN, and Fe⁻⁻⁻⁻⁴-NO bond lengths to the trans ligand identity. Interestingly, the Fe⁻⁻⁻⁻⁴-NO bond length is very similar for Fe⁻⁻⁻⁻⁴(TPP)(N-Melm)(NO) (1.74 Å) and Fe⁻⁻⁻⁻⁴(TPP)(4-methylpyridine)(NO) (1.72 Å), even though N-Melm and 4-methylpyridine are bases of different strengths. In contrast, the Fe⁻⁻⁻⁻⁴-CO bond distance is different in Fe⁻⁻⁻⁻⁴(deuterio)(THF)(CO) (1.706 Å) and Fe⁻⁻⁻⁻⁴(TPP)(pyridine)(CO) (1.77 Å).

Similarly, the steric trans effect which is observed for CO, O₂, and CN complexes of iron porphyrins is absent for the NO case. For example, the ν(Fe⁻⁻⁻⁻⁴-O₂) stretching frequency for solid Fe⁻⁻⁻⁻⁴-(TpivPP)(N-Melm)(O₂) appears 10 cm⁻¹ higher than that of the sterically hindered Fe⁻⁻⁻⁻⁴(TpivPP)(1,2-DiMelm)(O₂) complex, while the ν(Fe⁻⁻⁻⁻⁴-CN) stretching frequency is observed at 524 cm⁻¹ for both Fe⁻⁻⁻⁻⁴(OEP)(N-Melm)(NO) and Fe⁻⁻⁻⁻⁴(OEP)(1,2-DiMelm)(NO). Results from previous RRS studies of Mn⁻⁻⁻⁻⁴.

is significant in two different aspects: (1) First, a comparison of the \( \nu(\text{Fe}^{II}-\text{NO}) \) and \( \nu(\text{Fe}^{III}-\text{NO}) \) stretching frequencies in porphyrins and hemoproteins is now feasible. While \( \nu(\text{Fe}^{II}-\text{NO}) \) is observed \( \sim 30 \text{ cm}^{-1} \) lower in porphyrins than in hemoproteins, the effects of the protein on \( \nu(\text{Fe}^{III}-\text{NO}) \) are small. (2) Second, the detection of \( \nu(\text{Fe}^{II}-\text{NO}) \) in model porphyrins enabled us to study trans effects on this bond. The \( \text{Fe}^{II}-\text{NO} \) bond strength was determined to be independent of the electron-donating and stereochemical properties of the trans base. Clearly, the \( \text{Fe}^{II}-\text{NO} \) bond is unique in this respect, and future work may be directed toward understanding this system more fully.

Acknowledgment. We gratefully acknowledge Dr. C. K. Chang for providing some of the porphyrin samples, including heme-5, FeSP-13, FeSP-14, and FeSP-15.