

PROJECT ADMINISTRATION DATA SHEET



ORIGINAL



REVISION NO. _____

Project No. G-33-G09~~ORIGINAL~~DATE 7 / 6 / 84Project Director: Dr. Nai-Teng YuSchool ~~XXX~~ ChemistrySponsor: DHHS/PHS/NIH-National Institute of General Medical SciencesType Agreement: Grant No. 5 R01 GM18894-14Award Period: From 9/1/84 To 8/31/85 (Performance) 11/30/85 (Reports)Sponsor Amount: This Change Total to Date

Estimated: \$ _____ \$ _____

Funded: \$ 113,388 \$ 113,388Cost Sharing Amount: \$ 5,968 Cost Sharing No: G-33-348Title: "Laser-Excited Raman Spectroscopy of Biopolymers"

ADMINISTRATIVE DATA

OCA Contact Lynn Boyd x4820

1) Sponsor Technical Contact:

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2) Sponsor Admin/Contractual Matters:

Linda V. Roberts or D. O'DonovanGrants Management SpecialistNational Institute of Gen'l Medical
SciencesBethesda, MD 20014(301) 496-7746Defense Priority Rating: n/aMilitary Security Classification: n/a(or) Company/Industrial Proprietary: n/a

RESTRICTIONS

See Attached NIH Supplemental Information Sheet for Additional Requirements.

Travel: Foreign travel must have prior approval - Contact OCA in each case. Domestic travel requires sponsor approval where total will exceed greater of \$500 or 125% of approved proposal budget category.

Equipment: Title vests with GIT. We are accountable for all equipment purchased.

COMMENTS:

Follow-on to G-33-G08.14th year of continuing grant.

COPIES TO:

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Sponsor I.D. #02.108.001.84.026

GTRI
Library
Project File
Other Newton

SPONSORED PROJECT TERMINATION/CLOSEOUT SHEET

Date January 29, 1986Project No. G-33-G09School/~~EMX~~ ChemistryIncludes Subproject No.(s) N/AProject Director(s) Dr. Nai-Teng Yu~~GTGX~~ / GITSponsor DHHS/PHS/NIH-National Institute of General Medical SciencesTitle "Laser-Excited Raman Spectroscopy of Biopolymers"Effective Completion Date: 8/31/85 (Performance) 11/30/85 (Reports)

Grant/Contract Closeout Actions Remaining:

☐ None☒ ~~XXXXXXXXXX~~ Final Fiscal Report☐ Closing Documents☒ Final Report of Inventions 568 sent to project director☐ Govt. Property Inventory & Related Certificate☐ Classified Material Certificate☐ Other _____Continues Project No. G-33-G08Continued by Project No. G-33-G010

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SECTION IV PROGRESS REPORT SUMMARY		GRANT NUMBER GM 18894-15	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR Yu, Nai-Teng		PERIOD COVERED BY THIS REPORT	
NAME OF ORGANIZATION Georgia Institute of Technology		FROM 09/01/84	THROUGH 06/03/85
TITLE (Repeat title shown in item 1 on first page) Laser-excited Raman Spectroscopy of Biopolymers			

(SEE INSTRUCTIONS)

Publications:

1. Yu, N.-T., Benko, B., Kerr, E. A. and Gersonde, K. (1984) "Iron-Carbon Bond Lengths in Carbonmonoxy and Cyano-met Complexes of the Monomeric Insect Hemoglobin CTT III. A Critical Comparison between Resonance Raman and X-Ray Diffraction Studies" Proc. Natl. Acad. Sci., USA, 81, 5106-5110.
2. Kerr, E. A., Yu, N.-T. and Gersonde, K. (1984) "Assignment of the Fe-N (His) Stretching Mode in the Resonance Raman Spectra of a Monomeric Insect Cyanomet-hemoglobin" FEBS Lett., 178, 31-33.
3. Kerr, E. A., Yu, N.-T., Bartnicki, D.E. and Mizukami, H. (1985) "Resonance Raman Studies of CO and O₂ Binding to Elephant Myoglobin [Distal His(E7) → Gln] J. Biol. Chem. (in press)."
4. Kerr, E. A., Yu, N.-T., Gersonde, K., Parish, D. W. and Smith, K. M. (1985) "Iron-Histidine Stretching Vibration in the Deoxy State of Insect Hemoglobins with Different O₂ Affinities and Bohr-Effects " J. Biol. Chem. (submitted).
5. Kerr, E. A. and Yu, N.-T. (1986) "Vibrational Modes of Coordinates CO, CN⁻, O₂ and NO" in Biological Applications of Raman Spectroscopy" (Spiro, T. G., Ed.)² John-Wiley & Sons.

Reports

1. General Scientific Goals: No Change
2. Concise Description of Studies Conducted during the Budget Year, the Results obtained and their Significance.
 - (a) Detection of Fe-N (HIS) Stretching Vibration in Deoxy Complexes of Ferrous Adamantane Porphyrin Cyclophane with Sterically Unhindered Axial Bases.

Figure 1 shows the low frequency region (200-700 cm⁻¹) resonance Raman spectra of Fe(III) (oxidized) and Fe(II)(deoxy) adamantane porphyrin-6,6-cyclophane (APC) with N-MeIm as the axial base (see Fig. 2 for the chemical structure). The intense line observed at 214 cm⁻¹ is assigned to the Fe-N (N-MeIm) stretching vibration. For the picket fence complexes, the pent₅-coordinated Fe(II) hemes were favored by using 2-MeIM or 1,2-Me₂Im as an axial base. The bulky methyl group adjacent to the imidazole² nitrogen atoms provides steric hindrance to the formation of planar bis ligated complexes. For the adamantane heme, the bulky nature of the adamantane bridging group reduced binding of a second base. When 1,5-dicyclohexylimidazole was used as the axial base, titrations showed no evidence for

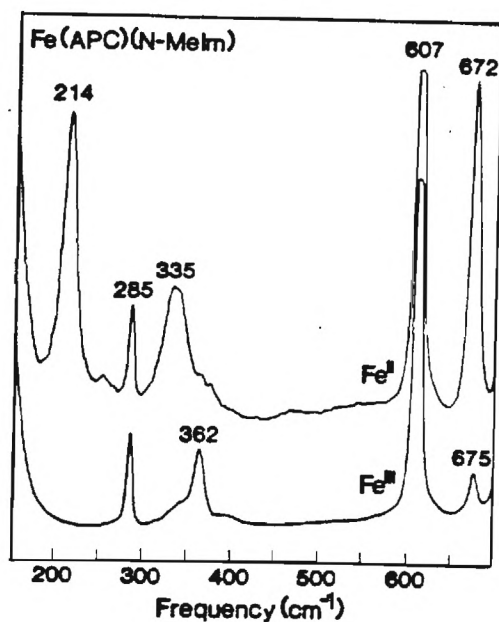
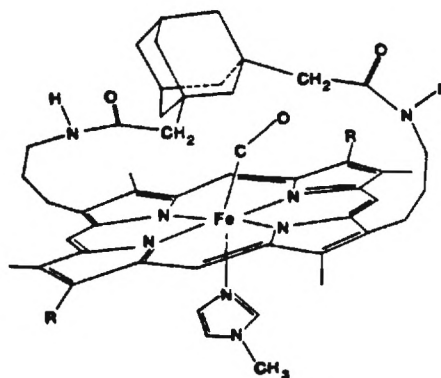


Fig. 1



hexacoordination. Unlike the Fe-N stretching vibration for Fe(II)(TpivPP)(N-Melm), the Fe-N stretching vibration for Fe(II)(APC)(N-Melm) is strong and easily observed. In addition, the $\nu(\text{Fe-N})$ at 214 cm^{-1} for APC is much lower than the 225 cm^{-1} value observed by Hori and Kitagawa for the $\nu(\text{Fe-N})$ of the "picket fence" porphyrin. It is also somewhat lower than the value of $\sim 220 \text{ cm}^{-1}$ observed in deoxy Hb and Mb.

(b) Resonance Raman Studies of Hemoglobins from the Liver parasite *Dicrocoelium dendriticum*.

The body fluid of the liver fluke *Dicrocoelium dendriticum* (DD), a flatworm that lives in the bile ducts of certain mammals, possesses a monomeric hemoglobin, functionally characterized to date. The distal which is perhaps the most primitive animal hemoglobin

histidine residue in DD Hb is absent and is replaced to an extreme degree by glycine. DD hemoglobin has the highest oxygen affinity reported to date for any monomeric hemoglobin i.e., $P_{1/2}(O_2) = 0.085$ mm Hg at pH 7.0 at 20°C. The lowest $P_{1/2}$ value reported is 0.0015 mm Hg for a Hb in the perienteric fluid of *Assacris lumbricoides*. This Hb, however, is octameric whereas DD Hb shows no cooperativity. Recently, monomeric DD Hb was shown to exhibit a relatively strong acid "Bohr effect" (K. Gersonde, personal communication). That is, it can exist in two O_2 affinity states, a high-affinity state at low pH and a low-affinity state at high pH. The transition between the two states is controlled by one Bohr-proton binding site. Of particular interest here is a resonance Raman investigation which can provide valuable information on the nature of the iron-ligand bonds in the DD Hb.

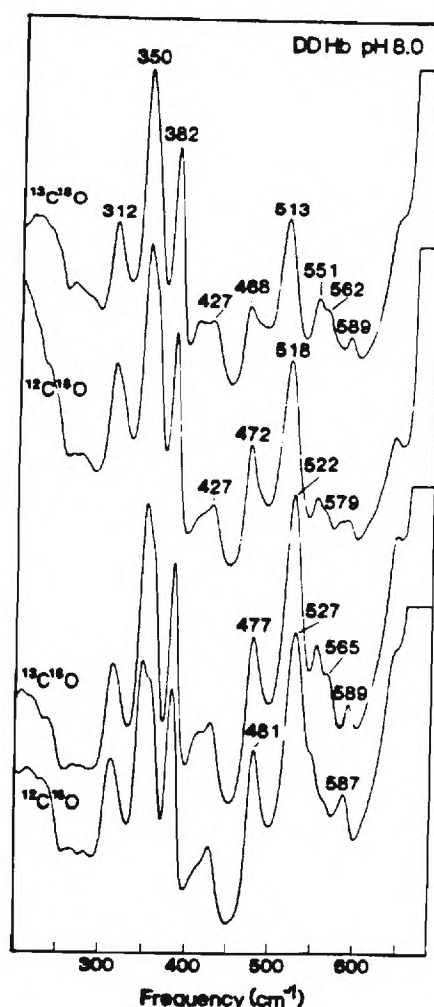


Fig. 3 shows the effects of carbon monoxide isotopic substitution on the 200-700 cm^{-1} region resonance Raman spectra of carbonmonoxy DD Hb at pH 8.0. Three isotope sensitive lines are observed at 481, 527 and 587

cm^{-1} . The lines at 481 cm^{-1} and 527 cm^{-1} exhibit a monotonous decrease in frequency with increasing CO mass; i.e., from 481 cm^{-1} ($^{12}\text{C}^{16}\text{O}$), 472 cm^{-1} ($^{12}\text{C}^{18}\text{O}$) and 468 cm^{-1} ($^{13}\text{C}^{18}\text{O}$) and from 527 cm^{-1} ($^{12}\text{C}^{16}\text{O}$) to 522 cm^{-1} ($^{13}\text{C}^{16}\text{O}$), 518 cm^{-1} ($^{12}\text{C}^{18}\text{O}$) and 513 cm^{-1} ($^{13}\text{C}^{18}\text{O}$), respectively. These isotope shift patterns clearly indicate that both lines are due to Fe-CO stretching vibrations. The isotope sensitive line at 587 cm^{-1} results from the overlap of two vibrations, an isotope sensitive one at $\sim 584 \text{ cm}^{-1}$ and an isotope insensitive one at 589 cm^{-1} . The isotope sensitive line at $\sim 584 \text{ cm}^{-1}$ is assigned to an Fe-C-O bending mode because of its characteristic "zigzag" shift pattern, that is, it shifts from $\sim 584 \text{ cm}^{-1}$ ($^{12}\text{C}^{16}\text{O}$) to $\sim 565 \text{ cm}^{-1}$ ($^{13}\text{C}^{16}\text{O}$), $\sim 579 \text{ cm}^{-1}$ ($^{12}\text{C}^{18}\text{O}$) and $\sim 562 \text{ cm}^{-1}$ ($^{13}\text{C}^{18}\text{O}$).

The observation of two strong Fe-CO stretching vibrations in the spectra of carbonmonoxy DD Hb is quite interesting. In fact, the two $\nu(\text{Fe-CO})$ frequencies of 527 cm^{-1} and 481 cm^{-1} are considerably higher and lower, respectively, than values detected previously for the $\nu(\text{Fe-CO})$ of other hemoproteins including 507 cm^{-1} for HbA, 517 cm^{-1} for sperm whale Mb, 516 cm^{-1} for elephant Mb, 500 cm^{-1} for CTT III, and 505 cm^{-1} for leg Hb. The structural implications of these $\nu(\text{Fe-CO})$ frequencies are now under careful studies.

(C) Resonance Raman Studies of Nitrosyl Cobalt Hemoproteins.

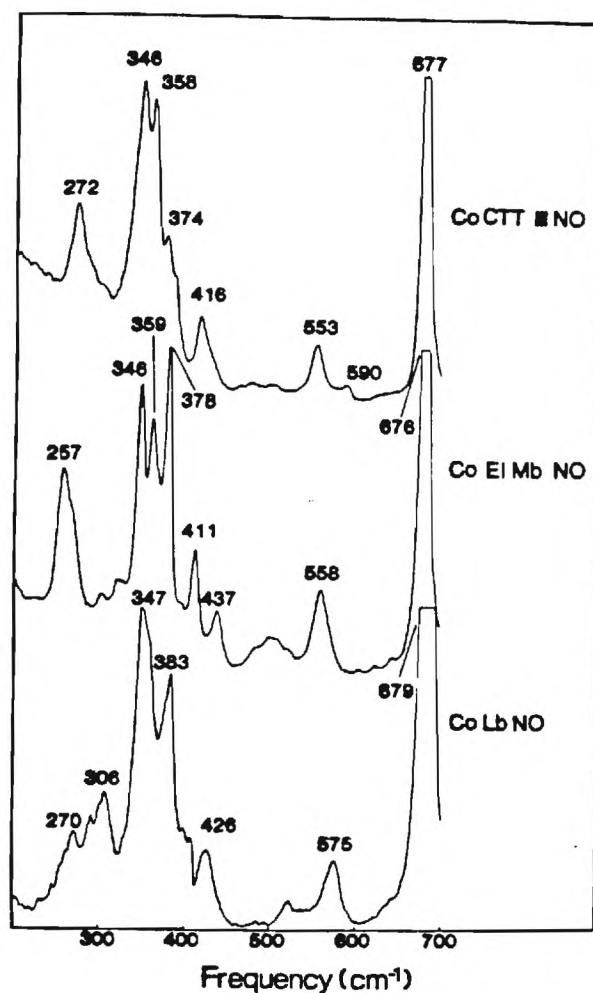


Fig. 4 shows the resonance Raman spectra of the nitrosyl complexes of cobalt CTT III, cobalt elephant Mb and cobalt leghemoglobin. If it is assumed that the main structural differences between these proteins lie in the nature of the heme pocket, then the absence or presence of the distal histidine apparently makes a differences in the frequency of the cobalt-nitrosyl vibration. In other words, cobalt sperm whole Mb and cobalt lb, both have a distal histidine and a $\nu(\text{Co-NO})$ at 573 cm^{-1} and 575 cm^{-1} , respectively. Cobalt elephant Mb and cobalt CTT do not contain a histidine in the distal position and have the $\nu(\text{Co-NO})$ at 558 cm^{-1} and 553 cm^{-1} . Further evidence is provided by the nitrosyl complex of cobalt "picket fence" porphyrin, which has its $\nu(\text{Co-NO})$ at 547 cm^{-1} and no distal amino acid interaction.

3. Specific Objective for the Coming Years:

- (i) To search for direct evidence for reciprocal changes in iron-axial ligand bonds in CO-ligated monomeric insert hemoglobins, induced by allosteric transition.
- (ii) To continue our studies on DD hemoglobin.
- (iii) Applications of the simple scheme for the estimation of metal-ligand bond length and geometry to carbonmonxy and cyano-met hemoproteins and heme complexes.
- (iv) To initiate the studies of CTT III complexes reconstitute with proto heme III; a symmetric heme; this hemoprotein does not have heme disorder.