15:40:59 OCA PAD INITIATION - PROJECT HEADER INFORMATION 08/15/89

Active

Project #: G-33-G14 Cost share #: Rev #: 0
Center # : 10/24-6-Q5169-4A0 Center shr #: OCA file #:
Work type : RES
Contract#: 5 R01 GM18894-19 (SEE COMMENT) Mod #: Document : GRANT
Prime #: Contract entity: GIT

Subprojects ? : N
Main project #:

Project unit: CHEM Unit code: 02.010.136
Project director(s):
YU N-T CHEM (404)894-4007

Sponsor/division names: DHHS/PHS/NIH / NATL INSTITUTES OF HEALTH
Sponsor/division codes: 108 / 001

Award period: 890901 to 900831 (performance) 901130 (reports)

Sponsor amount	New this change	Total to date
Contract value	173,050.00	173,050.00
Funded	173,050.00	173,050.00
Cost sharing amount		0.00

Does subcontracting plan apply ?: N

Title: LASER-EXCITED RAMAN SPECTROSCOPY OF BIOPOLYMERS

PROJECT ADMINISTRATION DATA

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Security class (U,C,S,TS) : U ONR resident rep. is ACO (Y/N): N
Defense priority rating : N/A NIH supplemental sheet
Equipment title vests with: Sponsor GIT X

Administrative comments -
INITIATION OF 19TH YEAR OF PROJECT. CONTINUATION OF G-33-G13.

SR 148-2

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

Closeout Notice Date 08/23/90

Project No. G-33-G14 Center No. 10/24-6-Q5169-4A0

Project Director YU N-T School/Lab CHEMISTRY

Sponsor DHHS/PHS/NIH/NATL INSTITUTES OF HEALTH

Contract/Grant No. 5 R01 GM18894-19 Contract Entity GIT

Prime Contract No.

Title LASER-EXCITED RAMAN SPECTROSCOPY OF BIOPOLYMERS

Effective Completion Date 900831 (Performance) 901130 (Reports)

Closeout Actions Required:	Y/N	Date Submitted
Final Invoice or Copy of Final Invoice	Y	
Final Report of Inventions and/or Subcontracts	N	
Government Property Inventory & Related Certificate	N	
Classified Material Certificate	N	
Release and Assignment	N	
Other	N	

Comments

Subproject Under Main Project No.

Continues Project No. G-33-G13

Distribution Required:

Project Director	Y
Administrative Network Representative	Y
GTRI Accounting/Grants and Contracts	Y
Procurement/Supply Services	Y
Research Property Managment	Y
Research Security Services	N
Reports Coordinator (OCA)	Y
GTRC	Y
Project File	Y
Other	N
	N

G-33-G14

SECTION IV PROGRESS REPORT SUMMARY	GRANT NUMBER EY01746-15	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR Yu, Nai-Teng	PERIOD COVERED BY THIS REPORT FROM	THROUGH
APPLICANT ORGANIZATION Georgia Institute of Technology	05/01/89	02/20/90

TITLE OF PROJECT *(Repeat title shown in item 1 on first page)*
Comparative Raman Studies of Human and Animal Lenses

(SEE INSTRUCTIONS)

1. The Plans for the Next Year of Support:

The specific aims for the next year of support are : (1) To continue FT-Raman studies of human brunescence cataracts with more sensitive Bruker FT-Raman spectrometer; (2) To study fluorescent lipid peroxidized products by the FT-Raman method; (3) To interpret the 406.7nm-excited fluorescence images of human lenses and compare them with those obtained at 350-365 nm excitation; (4) To continue our development of the techniques of near infrared-excited surface-enhanced Fourier-Transformed Raman spectroscopy for the detection of 3-OH-L-kynurenine-O-β-glucoside and its derivatives.

2. Concise Description of the Studies Conducted during the Current Budget Year:

a) *Development of New Technique: Near Infrared FT-Raman Spectroscopy for Cataractous Human Lenses*

We finally overcome the major difficulty in Raman spectroscopic studies of older and cataractous human lenses, especially the brunescent cataracts. These lenses exhibit high fluorescence with visible excitations. Previous attempts to obtain Raman spectra from senile cataractous lenses or normal human lenses older than 58 years were unsuccessful due to fluorescence interference. We now have obtained , for the first time, high quality Raman spectra of these lenses with a new technique: near infrared-excited Fourier transform (FT)-Raman spectroscopy. This technique employs excitation at 1.064 μm, of which the photon energy is too low to excite fluorescence. For the purpose of human lens studies, near-IR FT-Raman spectroscopy is definitely the best technique since it combines fluorescence rejection, in-situ applicability, and the multiplex / throughput advantages afforded by the Michelson interferometer over a conventional dispersive Raman spectrometer. The FT-Raman spectra can be further improved by the Bruker FT-Raman spectrometer that is much more sensitive than the Bomem DA3.02. Any lenses and their isolated constituents are now amenable for Raman studies.

b) *Surface-enhanced Raman Spectra of Eye Lens Pigments*

Surface-enhanced Raman spectroscopy (SERS) has been applied to study lenticular pigments that are present in the eyes of certain diurnally active animals. Using Ag hydrosols pre-aggregated with $NaClO_4$, we have obtained SERS spectra from dilute solutions of various model pigment compounds, including kynurenine, N-formylkynurenine, 3-hydroxykynurenine, β-carboline, bityrosine, anthranilic acid, 3-hydroxyanthranilic acid and oxindole. The results obtained from these model compounds show that SERS is a particularly sensitive technique for the identification of lens pigments. We also find a procedure that enables high-quality SERS data to be obtained for the yellow pigments in the lens homogenates of grey squirrels, ground squirrels and chipmunks. The surface Raman results confirm the identity of the low molecular weight, water soluble pigment in the grey squirrel lens as a derivative of 3-hydroxykynurenine, but reveal that lens pigmentation in ground squirrels and chipmunks involves new chromophores.

c) *Localization of UV-induced Changes in Mouse Lens*

We have compared the opacity produced by UV with that produced by X-ray in animal models. The first appearance of UV-induced cataract is in the deep cortical region and has essentially the same near-spherical symmetry as the lens itself. However, X-ray cataract appears in the posterior cortex. We reason that this difference in location must be due to procedural differences. X-rays are given as a short intense dose which is followed by a latent period of perhaps months before the opacity becomes apparent. The injured epithelial cells migrate from the anterior to the posterior where they appear as a posterior cataract. On the other hand, the UV dose is weak but long-continued so that the cataract produced represents the accumulation of injured cells all along the migration path of cells elongating as they become fiber cells. The oldest cells continue to receive radiation but at an intensity which continually decreases as they fall in the shadow of younger, newly irradiated cells. Thus the shape of the opacity is that of a near-sphere surrounding a core of clear fibers which were never irradiated as epithelial cells and surrounded by much younger cells which have not yet received enough radiation to produce a visible effect. We have obtained Raman evidence to support the above interpretation.

3. No change

4. Not Applicable

G-33-G14

SECTION IV PROGRESS REPORT SUMMARY	GRANT NUMBER GM 18894-20	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR Yu, Nai-Teng	PERIOD COVERED BY THIS REPORT FROM	THROUGH
APPLICANT ORGANIZATION Georgia Institute of Technology	09/01/89	06/05/90

TITLE OF PROJECT *(Repeat title shown in item 1 on first page)*

Laser-excited Raman Spectroscopy of Biopolymers

(SEE INSTRUCTIONS)

1. The Plans for the Next Year of Support:

The specific aims for the next year of support are : (1) To continue the development of a series of new techniques for the studies of biomolecules: *near IR-excited FT-Raman, near IR-excited FT surface-enhanced Raman, near IR-excited surface-enhanced hyper-Raman/resonance Raman*, and *hyper-resonance Raman*; (2) To study the pH-dependence of the two ν(Fe-CO) stretching vibrations at 527 and 481 cm^{-1} in liver fluke Dd hemoglobin; (3) To perform theoretical calculations on ligand modes based on FT-Raman spectra of metalloporohyrins and hemoproteins.

2. Concise Description of the Studies Conducted during the Current Budget Year:

a) *Development of New Technique: Near Infrared FT-Raman Spectroscopy of Photolabile Organocobalt B_{12} and Model Compounds*

We have taken a new initiative in developing the new technique, *near infrared-excited FT-Raman spectroscopy*, for the studies of photolabile and fluorescent biomolecules. For the first time, we reported (JACS, 111, 9256, **1990**) the stretching vibration of the Co-C bond in organocobalt B_{12} and model compounds. The FT-Raman spectra have been measured for a large number of B_{12} model compounds containing the $(DH)_2$ equatorial ligand system (DH = monoanion of dimethylglyoxime) in order to assess the importance of various factors (*viz.*, trans electronic effect, trans steric effect, environmental effect) that influence the Co-C bond stretch. The Co-CH_3 stretching mode in the solid state is generally detected as a very intense and sharp Raman line at ~ 500 cm^{-1}, which

exhibits a frequency decrease of 2-27 cm^{-1} in chloroform solution. Comparison of FT-Raman results with X-ray structural data indicates the existence of structural differences between the solid state and solution. It is suggested that the flexible $Co(DH)_2$ unit is bent from planarity more in solution than in the solid. Such a conformational distortion should lead to a weakening of the Co-C bond. Although there is a well-established relationship between Co-C bond cleavage rate of (4-X-pyridine)$Co(DH)_2R$ complexes and 4-X-pyridine basicity, FT-Raman studies of a series of (4-X-pyridine)$Co(DH)_2CH_3$ compounds (X = H, cyano, t-butyl and NMe_2) in chloroform unambiguously reveal the absence of a trans electronic influence on the Co-C stretching frequency in the ground state. A similar study of a series of $PR_3Co(DH)_2CH_3$ complexes (R = methyl, n-butyl, phenyl and cyclohexyl) confirms the presence of a trans steric influence. The observed trans steric influence in the ground state, however, is not large enough to account for the differences in Co-C bond cleavage rates for related B_{12} models with bulky axial alkyl groups. These findings suggest that the energetics of the transition state / ground state properties not directly related to Co-C bond strength are important. Since the ν(Co-C) stretch in these models is similar to that in the coenzyme, methyl B_{12}, there is a possibility that the B_{12}-dependent enzymes enhance the Co-C bond cleavage rate by lowering the overall energy of the transition state, rather than by significantly weakening the Co-C bond in the ground state.

b) ***Development of Surface-Enhanced Hyper-Raman Spectroscopy***

Hyper-Raman spectroscopy is another new technique that may have wide applications in studying biomolecules. With its relaxed selection rules, hyper-Raman technique is capable of providing new vibrational information. All IR-active vibrational modes are hyper-Raman allowed, and those modes inactive in both IR and Raman may be active in hyper-Raman

scattering. With a mode-locked Nd-YAG laser (1.064 μm, 82 MHz), we have demonstrated that, at constant average power, fiber-optic compression of the 100 pico-second fundamental output to 5 ps increases the intensities of surface-enhanced hyper-Raman scattering and second-harmonic generation by ~ one order of magnitude. We have obtained high-quality SEHRS spectra for two molecules (*viz.*, basic fuchsin and 3-hydroxykynurenine) adsorbed on Ag colloids with only 0.1 W laser excitation. We believe that it is possible now to develop the surface-enhanced hyper-Raman spectroscopy into a practical tool for investigating biological molecules.

In addition, we have demonstrated that the SEHRS effect can also be observed from molecules adsorbed on gold and copper colloidal surfaces, and that surface enhancements at the emitted hyper-Raman photon frequencies are not required for observing SEHRS signals.

c) ***The Effect of the Distal Residues on the Vibrational Modes of the Fe-CO Bond in Hemoglobin Studied by Protein Engineering***

Using an *Escherichia coli* gene expression system, we have engineered human hemoglobin (Hb) mutants having the distal histidine (E-7) and valine (E-11) residues replaced by other amino acids. The interaction between the mutated distal residues and bound carbon monoxide has been studied by Soret-excited resonance Raman spectroscopy. The replacement of Val-E11 by Ala, Leu, Ile and Met has no effect on the ν(C-O), ν(Fe-CO) stretching or δ(Fe-C-O) bending frequencies in both the α and β subunits of Hb, although some of these mutations affect the CO affinity as much as 40 fold. The strain imposed on the protein by the binding of CO is not localized in the Fe-CO bond and is probably distributed among many bonds in the globin. The replacement of His-E7 by Val or Gly brings the stretching frequencies ν(Fe-CO) and ν(C-O) close to those of free heme complexes. In contrast, the substitution of His-E7 by Gln, which is flexible and polar, produces no effects on the resonance Raman spectrum of either α or β globin. The replacement of His-E7 of b globin by Phe shows the same effect

as replacement by Gly or Val. Therefore the steric bulk of the distal residues is not the primary determinant of the Fe-CO ligand vibrational frequencies. The ability of both histidine and glutamine to alter the n(C-O, n(Fe-CO) or d(Fe-C-O) frequencies may be attributed to the polar nature of their side chains which can interact with bound CO in a similar manner.

d) ***Study of Active Site of Bovine Adrenocortical Cytochrome-450$_{11\beta}$ by Resonance Raman and EPR***

In collaborative with Dr. M. Tsubaki, we have obtained resonance Raman spectra of bovine adrenocortical cytochrome P-450$_{11\beta}$. The spectra revealed that the heme iron adopt pure penta-coordinated ferric high-spin state in contrast to the ferric high-spin cytochrome P-450scc-substrate complexes. In ferrous-CO state, a Fe-CO stretching mode was identified at 481.5 cm-1, very close to that of cytochrome P-450$_{scc}$ in cholesterol-complexed state (483 cm-1). The EPR spectra of the purified sample showed ferric high-spin signals (at g=7.98, 3.65 and 1.71) at 4.2 K, clearly distinct from those of cytochrome P-450scc ferric high-spin signals (g=8.06, 3.55 and 1.68). The EPR spectra of nitric oxide complex of ferrous cytochrome P-450$_{11\beta}$ showed EPR signals centered around g=2 having rhombic symmetry (g_x=2.068, g_z=2.000 and g_y=1.961) very similar to those of ferrous cytochrome P-450$_{scc}$-NO complex in the presence of 22(S)-hydroxycholesterol and 20(R), 22(R)-dihydroxycholesterol at 77 K. These spectral data indicate that the stereochemical structure surrounding the active site of cytochrome P-450$_{11\beta}$ bear a close resemblance to that of cytochrome P-450$_{scc}$ in ferrous ligated states (ferrous-CO and ferrous -NO states), but not in ferric high-spin state.

e) In collaboration with Dr. Denis Rousseau of AT&T Bell Laboratories, we have carried out resonance Raman studies of nitrosyl Mb at 20^{0}C and 77^{0}K. The isotope-sensitive Raman line at ~ 554 cm-1 does not change, indicating no dramatic change in ligand bonding geometry due to temperature decrease. Apparently, the EPR spectroscopic interpretation in the literature is incorrect.

3. No change

4. Not Applicable

5. **Publications:**

i) Nie, S., Marzilli, L.G., and Yu, N.-T. (**1989**) Near-Infrared Fourier Transform Raman Spectroscopy of Photolabile Organocobalt B_{12} and Model Compounds. 1. Detection of the Cobalt-Carbon Stretching Mode in the Solid State and in Solution. ***J. Am. Chem. Soc.*** *111*, 9256-58.

ii) Liu, H.-H., Lin, S.-H. and Yu, N.-T. (**1990**) "Resonance Raman enhancement of phenyl ring vibrational modes in phenyl iron complex of myoglobin" ***Biophys. J.*** *57*, 851-856.

iii) Nie, S., Lipscomb, Feng, S. and Yu, N.-T. (**1990**) Resonant and Nonresonant Surface-Enhanced Hyper-Raman Spectroscopy with a Picosecond Laser. Effect of the Excitation Pulse Width. ***Chem. Phys. Lett.*** 167, 35-40.

iv) Nie, S., Marzilli, P. A., Marzilli, L. G. and Yu, N.-T. (**1990**) Near Infrared Fourier Transform Raman Spectroscopy of Photolabile Organocobalt B_{12} and Model Compounds. Identification of the Co-C Bond Stretch in Cobalamins. ***J. Chem. Soc. Chem. Commun.*** (in press).

v) Nie, S., Marzilli, P.A., Marzilli, L. G. and Yu, N.-T. (**1990**) Near-Infrared Fourier Transform Raman Spectroscopy of Photolabile Organocobalt B_{12} and Model Compounds. 3. Vibrational Assessment of Factors Affecting the Co-C Bonds in Models. ***J. Am. Chem. Soc.*** (in press).

vi) Lipscomb, L. A., Nie, S., Feng, S. and Yu, N.-T. (**1990**) Surface-Enhanced Hyper-Raman Spectroscopy with a Picosecond Laser: Gold and Copper Colloids. ***Chem. Phys. Lett.*** (in press).

vii) Lin, S.-H., Yu, N.-T., Tame, J., Shih, D., Renaud, J.-P., Pagnier, J. and Nagai, K. (**1990**) " The Effect of the Distal Residues on the Vibrational Modes of the Fe-CO Bond in Haemoglobin Studied by Protein Engineering" ***Biochenistry*** (in press).

viii) Tsubaki, M., Ichikawa, Y., Yoshikawa, S., Fujimoto, Y., Yu, N.-T. and Hori, H. (**1990**) "Active Site of Bovine Adrenocortical Cytochrome P-$450_{11\beta}$ Studied by Resonance Raman and Electron Paramagnetic Resonance Spectroscopies: Distinction from Cytochrome P-450_{scc}" ***Biochemistry*** (in press).