Project #: G-33-G14  Cost share #:  
Center #: 10/24-6-Q5169-4A0  Center shr #:  
Contract#: 5 R01 GM18894-19 (SEE COMMENT) Mod #:  
Prime #:  
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  
Sponsor/division codes: 108  
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  

OCA contact: Kathleen R. Ehlinger 894-4820  
Sponsor technical contact  
DR. HELEN SUNSHINE, PROGRAM ADMIN. (301)496-7309  
NatlInst of General Medical Science National Institutes of Health Bethesda, MD 20892  
Security class (U,C,S,TS) : U  
Defense priority rating : N/A  
Equipment title vests with: Sponsor  
ONR resident rep. is ACO (Y/N): N  
NIH supplemental sheet GIT X  

Administrative comments -  
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/NATIONAL 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:

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<th>Action</th>
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<tr>
<td>Final Invoice or Copy of Final Invoice</td>
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Comments

Subproject Under Main Project No.

Continues Project No. G-33-G13

Distribution Required:

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

Y
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N

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 brunescence 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 NaClO₄, we have obtained SERS spectra from dilute solutions of various model pigment compounds, including kynurenic acid, 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-hydroxykynurenic acid, 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
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 metalloporphyrins 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 B₁₂ 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 B₁₂ and model compounds. The FT-Raman spectra have been measured for a large number of B₁₂ 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-CH₃ 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)$_2$R complexes and 4-X-pyridine basicity, FT-Raman studies of a series of (4-X-pyridine)Co(DH)$_2$CH$_3$ 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$_3$Co(DH)$_2$CH$_3$ 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 v(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 β 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\textsubscript{11β} by Resonance Raman and EPR**

In collaborative with Dr. M. Tsubaki, we have obtained
resonance Raman spectra of bovine adrenocortical cytochrome
P-450\textsubscript{11β}. The spectra revealed that the heme iron adopt pure
penta-coordinated ferric high-spin state in contrast to the
ferric high-spin cytochrome P-450\textsubscript{scc}-substrate complexes. In
ferrous-CO state, a Fe-CO stretching mode was identified at
481.5 cm\textsuperscript{-1}, very close to that of cytochrome P-450\textsubscript{scc} in
cholesterol-complexed state (483 cm\textsuperscript{-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-450\textsubscript{scc} ferric high-spin signals (g=8.06, 3.55 and 1.68). The
EPR spectra of nitric oxide complex of ferrous cytochrome P-
450\textsubscript{11β} showed EPR signals centered around g=2 having
rhombic symmetry (g\textsubscript{x}=2.068, g\textsubscript{z}=2.000 and g\textsubscript{y}=1.961) very
similar to those of ferrous cytochrome P-450\textsubscript{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\textsubscript{11β} bear a close resemblance to that of
cytochrome P-450\textsubscript{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°C and 77°K. The isotope-sensitive Raman
line at ~ 554 cm\textsuperscript{-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.


