Title: Comparative Raman Studies of Human and Animal Lenses

Type Agreement: Grant 5R01-EY01746-10

Award Period: From 5/1/85 To 4/30/86 (Performance) 7/31/86 (Reports)

Sponsor Amount:
- Estimated: $108,135
- Funded: $108,135

Cost Sharing Amount: $5,894

Title: Comparative Raman Studies of Human and Animal Lenses

COMMENTS:
No funds may be expended after 4/30/86
SPONSORED PROJECT TERMINATION/CLOSEOUT SHEET

Date 9/19/86

Project No. G-33-A10

School/Dept. Chem.

Includes Subproject No.(s) N/A

Project Director(s) N. Yu

Sponsor DHHS/PHS/KTH/NET

Title Comparative Raman Studies of Human and Animal Lenses

Effective Completion Date: 4/30/86 (Performance) (Reports)

Grant/Contract Closeout Actions Remaining: Reporting to be done under G-33-A11.

- [ ] None
- [XX] Final Invoice or Final Fiscal Report
- [ ] Closing Documents
- [ ] Final Report of Inventions
- [ ] Govt. Property Inventory & Related Certificate
- [ ] Classified Material Certificate
- [ ] Other

Continues Project No. G-33-A09

Continued by Project No. G-33-A11

PIES TO:

- Project Director
- Administration Network
- Property Management
- Accounting
- GTRI Supply Services
- Security Services
- Coordinator (OCA)

Library
GTRC
Research Communications (2)
Project File
Other

T. Newton
A. Jones
R. Embry

RM OCA 69.265
SECTION IV
PROGRESS REPORT SUMMARY

PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR
Yu, NAI-TENG

NAME OF ORGANIZATION
Georgia Institute of Technology

TITLE
Comparative Raman Studies of Human and Animal Lenses

FROM
05/01/85

THROUGH
03/01/86

GRANT NUMBER
EY01746-11

Progress Report:

1. General Scientific goals of the project during the budget year: no change.

2. Concise description of the progress:

(a) We have completely automated our conventional Raman spectrometer which allows us to average signals from multiple scans; thus greatly enhancing the signal-to-noise in the spectra. Special techniques have also been developed to obtain high-quality Raman spectra of cataractous human and animal lenses. Such studies have been hindered previously by poor signal-to-noise.

(b) The Spex model 1870 monochromator used in our Raman/fluorescence microprobe surface scanning system has been replaced by a Spex Triplemate which has a much better stray light rejection. The operation of this system has been delayed.

(c) We have just published a paper entitled "Disulfide bond formation in the eye lens" in Proc. Natl. Acad. which has attracted the attention of Prof. S. Varma who considers our findings about the constancy of G-S-S-G in the human lens as "indeed very novel" (see attached letter).

(d) We have carried out near UV photolysis experiments. Normal age-matched guinea pig lenses were compared to those exposed to (i) long term near UV -9 months in vivo and (ii) short term near UV (3.5 hours) in vitro from a He-Cd laser at 325 nm. Tryptophan and fluorescence along the visual axis (VA) were obtained using the Raman optical dissection technique. The fluorescence profiles (Excitation/Emission = 457.9/497 nm) indicate that the major alteration by UV was in the nucleus with the least in the posterior cortex. Normal aging lenses had no apparent change in the tryptophan profile between 3 days and 12 months. The UV-irradiated lenses also showed no appreciable differences from normal aging patterns. These results indicate there is no apparent tryptophan photolysis in the guinea pig lens by long-wave ultra-violet light.

(e) We have detected the S-S bond stretching vibration from the mixed disulfides (γ-glutathione and γ-mercaptoethanol) at 510 cm\(^{-1}\) (see Fig. 1). The absence of a disulfide vibration in γ-crystallin (both in aqueous solution and in lyophilized state (see Fig. 2) indicates that the seven thiol groups in this protein are resistant to air oxidation and capable of maintaining their reduced state in the absence

Two copies of each are provided with this application.
of added reducing agents during isolation. Upon titration with 5 equivalents of p-hydroxymercuribenzoate, a strong Raman line was detected at 342 cm$^{-1}$, which is attributed to the Hg-S stretching vibration of the mercaptide complex. On the other hand, the S-H vibration region (2500-2700 cm$^{-1}$) exhibits two resolved peaks at 2562 and 2580 cm$^{-1}$ with an intensity ratio of 2/5. The two cysteine residues at positions 18 and 78, which are inaccessible to p-hydroxymercuribenzoate, was found to make contributions to Raman intensity at 2580 cm$^{-1}$. The two most accessible thiol groups (possibly at positions 15 and 22) also give rise to the S-H vibration at 2580 cm$^{-1}$.

We have obtained Raman spectra of two Tibet human cataractous lenses (44- and 51-year old), which exhibit unusually low level of total sulfhydryl. Variations of fluorescence maximum as a function of excitation wavelength have also been obtained.

3. Specific Objectives for the Coming Year:

(a) Studies of Tibet human cataractous lenses by Raman/fluorescence techniques.

(b) Construct the S-H and S-S profiles along the visual axis of Tibet bovine (Yak) lenses ranging from 4 to 18-year old.

(c) Comparison of Raman spectra between age-matched Emory mouse lens and cataract-resistant mouse lens in the entire spectral region (100-4,000 cm$^{-1}$).

(d) Raman studies of crystallins from very young rat lenses (<20 days) before the occurrence of significant protein oxidation (S-S formation). Comparison of crystallins from rat and bovine lenses should provide useful information about the possible differences in the 3-dimensional distributions of -S-H groups.