

PROJECT ADMINISTRATION DATA SHEET

ORIGINAL REVISION NO. _____

Project No. G-33-A10 (Q5250-0A0) ~~XXXX~~GIT DATE 6 / 4 / 85

Project Director: N. Yu School/ Lab XXX Chem. _____

Sponsor: DHHS/PHS/NIH/NEI

Type Agreement: Grant 5R01-EY01746-10

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

Sponsor Amount: This Change Total to Date

Estimated: \$ _____ \$ 108,135

Funded: \$ _____ \$ 108,135

Cost Sharing Amount: \$ 5,894 Cost Sharing No: G-33-387

Title: Comparative Raman Studies of Human and Animal Lenses

ADMINISTRATIVE DATA OCA Contact John Schonk x4820

1) Sponsor Technical Contact: Henry N. Fukui 2) Sponsor Admin/Contractual Matters: Frances M. Goff

National Institutes of Health National Institutes of Health

National Eye Institute National Eye Institute

Bethesda, MD 20205 Bethesda, MD 20205

301/496-5884

Defense Priority Rating: N/A Military Security Classification: N/A

(or) Company/Industrial Proprietary: N/A

RESTRICTIONS

See Attached N/A 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

COMMENTS:

No funds may be expended after 4/30/86



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SPONSORED PROJECT TERMINATION/CLOSEOUT SHEET

SR 74

Date 9/19/86

Project No. G-33-A10 School/~~Eng~~ Chem.

Includes Subproject No.(s) N/A

Project Director(s) N. Yu ~~GPRC~~ / GIT

Sponsor DHHS/PHS/NIH/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
- 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

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SECTION IV
PROGRESS REPORT SUMMARY

GRANT NUMBER

EY01746-11

PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR

Yu, NAI-TENG

PERIOD COVERED BY THIS REPORT

FROM

05/01/85

THROUGH

03/01/86

NAME OF ORGANIZATION

Georgia Institute of Technology

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

Comparative Raman Studies of Human and Animal Lenses

(SEE INSTRUCTIONS)

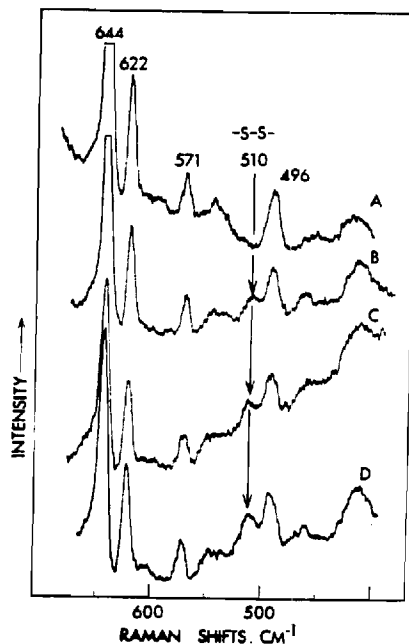
- Publications:
1. Yu, N.-T., DeNagel, D. C., Pruett, P. L. and Kuck, J.F.R. (1985) "Disulfide Bond Formation in the Eye Lens" Proc. Natl. Acad. Sci. USA, 82, 7965-7968.
 2. Barron, B. C., Yu, N.-T. and Kuck, J.F.R., Jr. (1986) "UV and Aging Effects on Tryptophan along the Visual Axis of Intact Guinea Pig Lenses" Invest. Ophthalmol. Vis. Sci. (to be submitted).

Two copies of each are provided with this application.

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 kaser 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_{II} -glutathione and γ_{II} -mercaptoethanol) at 510 cm^{-1} (see Fig. 1). The absence of a disulfide vibration in γ_{II} -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

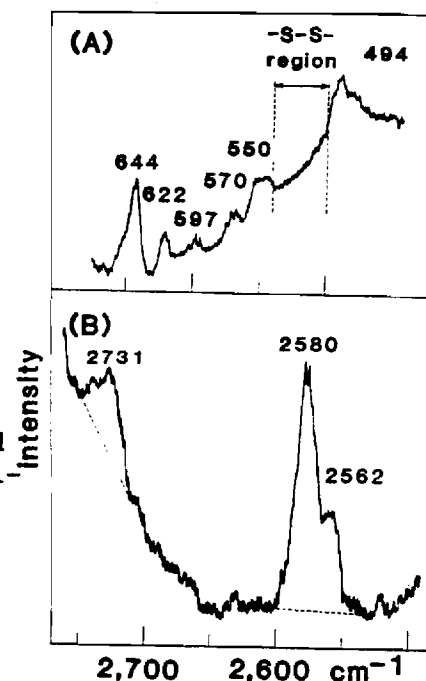
of added reducing agents during isolation. Upon titration with 5 equivalents of p-hydroxymercuribenzoate, a strong Raman line was detected at 342 cm^{-1} , which is attributed to the Hg-S stretching vibration of the mercaptide complex. On the other hand, the S-H vibration region ($2500\text{--}2700\text{ cm}^{-1}$) 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} .



<---Figure 1.

- (A) γ_{II} crystallin
- (B) γ_{II} -crystallin-mercaptoethanol adduct
- (C) γ_{II} crystallin-glutathione adduct
- (D) γ_{II} crystallin-(glutathione)₂ adduct

Figure 2.-->
Raman spectra (in the S-S and S-H regions) calf γ_{II} crystallin in aqueous solution.



(f) 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 ($100\text{--}4,000\text{ cm}^{-1}$).
- (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.