GEORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

PROJECT ADMINISTRATION DATA SHEET

[X] ORIGINAL [ ] REVISION NO. _____

Project No./(Center No.) G-33-618 R6307-1A0 GTRC/GIT DATE 6 / 24 /87

Project Director: Dr. R.F. Borkman School/Lab Chemistry

Sponsor: DHHS/PHS/NIH/National Eye Institute

Agreement No.: Grant No. 1 R01 EY06800-01

Award Period: From 5/1/87 To 4/30/88 (Performance) 7/31/88 Reports

Sponsor Amount:	New With This Change	Total to Date
Contract Value: $		$ 138,454
Funded: $		$ 138,454

Cost Sharing No./(Center No.) G-33-327(F6307-1A0) Cost Sharing: $ 8,620

Title: Photochemistry and Spectroscopy of Lenses and Lens Proteins

ADMINISTRATIVE DATA OCA Contact E. Faith Gleason x4-4820

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Military Security Classification: N/A
(or) Company/Industrial Proprietary: _____

ONR Resident Rep. is ACO: _____ Yes X No
Defense Priority Rating: _____

RESTRICTIONS

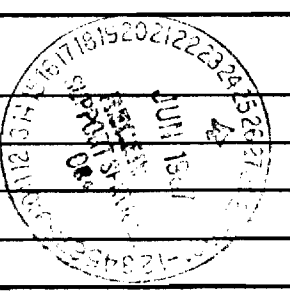
See Attached _____ Supplemental Information Sheet for Additional Requirements.

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:

This grant has been recommended for support for 5 years.



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GEORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION/CLOSEOUT SHEET

Date 6/24/88

Project No. G-33-618 School/Lab Chemistry

Includes Subproject No.(s) N/A

Project Director(s) R. F. Borkman GTRC/GIT

Sponsor DHHS/PHS/NIG/NATIONAL EYE INSTITUTE

Title Photochemistry and Spectroscopy of Lenses and Lens Proteins

Effective Completion Date: 4/30/88 (Performance) N/A (Reports)

Grant/Contract Closeout Actions Remaining:

[XX] None

[ ] Final Invoice or Copy of Last Invoice Serving as Final Already submitted.

[ ] Release and Assignment

[ ] Final Report of Inventions and/or Subcontract:
Patent and Subcontract Questionnaire sent to Project Director [ ]

[ ] Govt. Property Inventory & Related Certificate

[ ] Classified Material Certificate

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Continues Project No. Continued by Project No. G33-633

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G-39-617

SECTION IV **PROGRESS REPORT SUMMARY**	GRANT NUMBER EY-6800-02	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR Raymond F. Borkman	PERIOD COVERED BY THIS REPORT FROM	THROUGH
APPLICANT ORGANIZATION Georgia Institute of Technology	5-1-88	4-30-89

TITLE OF PROJECT *(Repeat title shown in item 1 on first page)*
Photochemistry & Spectroscopy of Lenses & Lens Proteins

(SEE INSTRUCTIONS)

1. RESEARCH PLANS FOR NEXT YEAR

a. Specific Aims:

A brief summary of the specific aims of the project, as stated in the original proposal, is provided here along with any current plans to update or modify these aims.

i. To determine average tyrosine-to-tryptophan distances in the lens crystallin proteins by fluorescence energy transfer experiments. This goal remains unchanged, but is not a high priority aim for the coming year since we are in the process of adapting a computer interface to our fluorescence spectrometer. We have one paper [1] in this area previously, and when the spectrometer interface has been added, and the software tested, we will be in a better position to continue these measurements, more precisely and reliably than is currently feasible. Thus, we will return to this part of the project when the new apparatus is ready. This should occur sometime in the latter part of the coming project year.

ii. To determine the local environments of tryptophan residues in lens crystallins using acrylamide quenching of protein fluorescence. A great deal has already been accomplished on this aim, and two papers are now in print [2,3] dealing with acrylamide quenching of fluorescence in the calf lens crystallins: alpha, beta, and gamma II, III, IV. As a supplemental aim, we are presently attempting to elucidate the molecular mechanism of acrylamide quenching of tryptophan fluorescence and tryptophan photochemistry, and completion of this will be a high priority goal in the coming year.

iii. To determine the anatomical distribution of photochemical damage in whole lenses exposed to UV laser radiation of various wavelengths. The first results using the excimer laser source (funds provided by this grant) are just beginning to become available. We have not yet determined any damage distributions, but we have demonstrated that this laser can produce measurable light scattering changes in whole bovine lenses for exposure times as short as 10 - 15 minutes. We will be continuing to pursue this topic in the coming year.

iv. To determine specific sites (amino acid sequence numbers) where photochemical damage occurs in lens crystallin proteins using tryptic digestion and HPLC. This topic is being very actively pursued and we will continue to do so. In the coming year we will be able to monitor the photo-chemical loss of specific tryptophan residues in the gamma-II sequence, for a variety of wavelength and sensitizer conditions. Significant progress on this aim is a very high priority goal for the coming year.

v. To determine mechanisms of action of UV radiation on whole lens and lens proteins using a variety of analysis techniques including light scattering. This continues to be an active area of research in the PI's lab. We have a publication in preparation, and will extend these studies during the coming year. In particular, we hope to be able to relate the photochemical reactivities of the gamma-II, III, and IV calf crystallins to their molecular structures. This will be an active area for the coming year.

b. Experimental Design and Methods:

The work associated with specific aims (i), (iii), (iv), and (v) is not altered relative to the original proposal, and hence no new procedures are introduced. For aim (ii) we have added the supplemental goal of obtaining data relevant to the mechanism of acrylamide quenching. We plan to measure Stern-Volmer quenching rate constants, Ksv, for fluorescence, photolysis, and electron ejection and to compare the values. Equal values will be interpreted to mean that all three quenching processes likely proceed through the same excited state intermediate (perhaps a charge-transfer complex between tryptophan and acrylamide). Dissimilar values of Ksv, for quenching the three photoprocesses, will be taken as evidence of independent quenching pathways (not involving a common intermediate). For these additional studies we propose methodology as follows:

We require the magnitudes of Stern-Volmer quenching rate constants for three processes: fluorescence, photolysis, and electron ejection. Of these, the method of obtaining Ksv for fluorescence quenching is well-known and was described in our original proposal and earlier publications [2,3]. The second process, acrylamide quenching of photolysis, requires us to obtain a reaction rate constant for several tryptophan solutions, of varying acrylamide concentration, and then prepare a Stern-Volmer plot of rate constant versus acrylamide concentration. Solutions of 0.1 mM tryptophan containing added acrylamide concentrations in the range 0.05 to 0.3 M will be prepared, and 1.0 ml samples will be photolyzed at 295 nm in a 1.0 cm path quartz cuvette. Four aliquots will be taken for each acrylamide concentration and these will be photolyzed for periods of 30, 60, 90, and 120 minutes. The solutions

will then be analyzed using reversed phase HPLC with a VYDAC column--flow rate of 1.0 ml/min, eluted with a methanol/water gradient (10-20% methanol). The chromatograms are monitored for loss of tryptophan starting material. The rate constants for Trp loss, k, are obtained as the slopes of plots of log(Co/C) versus time, where C and Co are the tryptophan concentrations at time t and time 0 respectively. Finally, the k values are plotted as ko/k versus [Q], where k and ko are the photolysis rate constants for acrylamide concentration 0 and [Q], and [Q] is the molar acrylamide concentration. The Stern-Volmer constant, Ksv, is the slope of this (linear) plot.

To obtain the Stern-Volmer quenching constant for photoelectron ejection in tryptophan, we are collaborating with Dr. Jay Huebner of the University of North Florida in Jacksonville, FL. For several years, Dr. Huebner has been developing instrumentation for measuring trans-membrane voltages induced by photochemical intermediates [4]. He has shown that tryptophan gives a voltage transient signal in his apparatus which may be attributable to electron photoejection. It was arranged for Dr. Huebner to spend the Fall, 1987 academic quarter in Dr. Borkman's lab at Georgia Tech and to collaborate on measuring the possible acrylamide quenching of the voltage transients observed previously by Dr. Huebner. The necessary measurements were made, and we are now in the process of analyzing the data and relating it to the fluorescence and photolysis quenching experiments being done in the PI's lab.

2. SUMMARY OF STUDIES--CURRENT YEAR:

This progress report will be organized around the five major aims cited above and in the original proposal.

i. An IBM PS/2 model 30 computer has been purchased and equipped with a DAS-16F analog-to-digital interface board. This will be used to control our fluorescence spectrometer and to digitize and store spectral data. The data will be analyzed and processed using the LABTECH NOTEBOOK software system which has also been purchased. Once this system has been implemented, we will use it to resume work on the fluorescence energy transfer studies of tyrosine-tryptophan distances in the lens crystallin proteins, calf gamma-III and gamma-IV. Calf gamma-II was done earlier [1].

ii. Considerable progress has been made in the use of the fluorescence quenching method to determine the local environments of tryptophan residues in the calf gamma crystallin subfractions gamma-II, -III, and -IV. The main conclusion is that the Trp residues in gamma-IV crystallin are the most exposed to solvent of this group of proteins; gamma-II Trps are the least exposed, and those of gamma-III

are intermediate. The differences are very large indeed. The rate constant for quenching the tryptophan fluorescence in gamma-IV crystallin is at least six times larger than that for quenching in gamma-II crystallin. This work has now appeared in print [3] and the conclusions agree with those from Chakrabarti's lab [5], based on other kinds of spectroscopic measurements. A summary of quenching rate constant values for the calf gamma crystallin subfractions II, III, and IV is given in Table 1 of the APPENDIX.

iii. We have only done a few photodamage experiments on whole lenses to date. The apparatus used for these studies allowed us to monitor the transmission of visible light as a function of time of exposure to UV laser radiation. The radiation source in these studies is our new LUMONICS excimer laser, purchased with funds from this grant. This laser can output radiation at wavelengths of 351 and 308 nm which are of particular interest for lens studies. The apparatus used in these particular experiments is analogous to that shown in Fig. 1, except that it is operated in the transmission mode rather than the right-angle scattering mode for these studies. Some typical data are shown in Fig. 2, which depicts the visible light transmission of a whole calf lens exposed to 308 nm excimer laser radiation for up to 50 minutes. The data show that the optical density of the lens increased very substantially during the first 15-20 minutes of irradiation and then leveled off at a constant value for the remaining irradiation period. These short experimental times are very important since they reduce the amount of time the lens must be kept in a viable condition, in vitro. Such experiments would not be possible without the excimer laser. SDS-PAGE analysis of proteins extracted from irradiated whole lenses showed evidence for protein crosslinking. After the preliminary work, showing the feasibility of whole lens irradiation studies has been completed, we will begin to collect data on anatomical distributions of damage in UV irradiated whole lenses. This will be done as shown in Fig.3, which depicts dissection of a whole lens, with separation into anterior cortex, nucleus, and posterior cortex prior to SDS-PAGE and other analyses.

iv. This topic is being very actively pursued and we will continue to do so in the coming year. A key item of instrumentation, a fluorescence detector for HPLC, has been purchased with funds from this grant and has been incorporated into a borrowed HPLC system. A reversed phase column for separating tryptic digest fragments was also purchased. We have established digestion conditions and have chromatographically resolved, and selectively detected using fluorescence detection, the tryptophan-containing tryptic fragments from UV irradiated gamma-II crystallin in buffer solution, see Fig. 4. In this way we are able to monitor the photo-chemical loss of specific tryptophan

residues in the gamma-II sequence, for a variety of wavelength and sensitizer conditions.

v. We have already completed, and are preparing for publication, an extensive study of photolysis of the calf gamma crystallin subfractions, gamma-II, III and IV. The irradiations were done using a 337.1 nm nitrogen laser or a xenon arc lamp/monochromator system at 290 nm. The protein solutions were irradiated both with and without the photosensitizers N-formylkynurenine (NFK) and riboflavin. Some of the data are summarized in Figs. 5 and 6. We found that the gamma-IV fraction was most susceptible to photochemical production of light scattering, both with and without sensitizers. The effect of added sensitizers was to increase the rates of scatter production, by a factor of 3-5 in the case of NFK, and by a factor of up to 50 in the case of riboflavin. The UV irradiations were found to cause significant protein insolubilization. Centrifugation of gamma crystallin solutions after UV treatment resulted in collection of a precipitate and a clear supernatant. Analysis of the supernatant by BIORAD protein assay showed loss of up to 50 % of the original soluble protein. When the UV irradiated protein solutions were analyzed by fluorescence we also found that the gamma-IV fraction showed the greatest photoreactivity. It is interesting to note that gamma-IV, the most photochemically active of the calf gamma crystallins, was determined in our above-described acrylamide fluorescence quenching studies to have the most solvent-exposed tryptophans. It is not yet clear whether there is a causal connection between these two observations. We have used SDS-PAGE to determine the degree of photo-crosslinking in the various calf gamma subfractions. We find evidence for higher molecular weight aggregates, at 40,000 and 60,000 dalton and at very high molecular weight (did not enter the gels), following UV exposure of dilute (1.0 mg/ml) gamma crystallin solutions.

3. HUMAN SUBJECTS

None planned for this year.

4. VERTEBRATE ANIMALS

No changes are anticipated relative to the original proposal. The only use is of rat or calf eyes in vitro. The former animals are obtainable from commercial suppliers and are sacrificed upon receipt. The calf lenses are from whole globes provided by a local slaughterhouse.