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NOTICE OF PROJECT CLOSEOUT

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Project Director R. F. Borkman School/Lab Chem.

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Title Photochemistry & Spectroscopy of Lenses & Lens Proteins

Effective Completion Date 4/30/89 (Performance) 7/31/89 (Reports)

Closeout Actions Required:

____ None
X Final Invoice or Copy of Last Invoice
____ Final Report of Inventions and/or Subcontracts
____ Government Property Inventory & Related Certificate
____ Classified Material Certificate
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____ Other ____

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Continues Project No. G-33-618 Continued by Project No. ____

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G-33-63

SECTION IV **PROGRESS REPORT SUMMARY**	GRANT NUMBER EY-6800-03	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR Raymond F. Borkman	PERIOD COVERED BY THIS REPORT FROM	THROUGH
APPLICANT ORGANIZATION Georgia Institute of Technology	5-1-89	4-30-90

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. The purpose of these experiments was to provide protein structure information for the lens crystallins. Our work on Gamma-II crystallin protein was published earlier [1]. However, it now appears that X-ray structures for several of the Gamma-III and Gamma-IV proteins, as well as for some of the Beta crystallins, will soon be available in print [Blundell, personal communication]. Since the X-ray data will provide more complete structural information than our proposed energy transfer work, we are awaiting the X-ray results at the present time. Our experiments can be activated at any future time if it seems appropriate to do so. For example, if X-ray data prove to be unobtainable for particular crystallin proteins, these will be suitable candidates for energy transfer studies.

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 we have two papers in print [2,3], dealing with acrylamide quenching of Trp fluorescence in the calf lens crystallins: alpha, beta, and gamma II, III, IV. Papers from other laboratories, which use the acrylamide quenching approach--first applied to lens protein structures in our laboratory, have now appeared [4,5,6]. An auxiliary study of acrylamide quenching of free Trp monomer fluorescence, photochemistry, and photoionization has also been completed and is in press [7]. No further studies in this area are planned for the coming year.

iii. To determine the anatomical distribution of photo-chemical damage in whole lenses exposed to UV laser radiation of various wavelengths. Results of photodamage experiments on whole calf lenses, using the excimer laser source purchased with funds provided by this grant, are steadily accumulating in our laboratory, and we plan to continue to pursue this during the coming project 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 actively

pursued and we will continue to do so. Both the photochemical loss of specific tryptophan residues in the gamma-II sequence, and the site(s) of photochemical crosslinks are being sought, for a variety of wavelength and sensitizer conditions. Significant progress has already been made on this aim, and it has a high priority for next 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 for us. We have two publications in preparation [8,9], and will extend these studies during the coming year. An area of increased emphasis is photochemical studies on lens homogenates and comparison with photochemical behavior of individual lens crystallin proteins. During the past year we have relied increasingly on SDS-PAGE analysis of UV irradiated lens proteins, and thus we have used project funds to purchase our own apparatus for these experiments. We ultimately hope to be able to reconstruct the behavior of lens homogenates (SDS-PAGE patterns and light scattering) in terms of the photochemical behavior of the individual crystallin and membrane proteins.

b. Experimental Design and Methods:

The proposed current work associated with specific aims (iii), (iv), and (v) does not require the introduction of any new experimental procedures beyond those given in the original proposal.

2. SUMMARY OF STUDIES--CURRENT YEAR:

This progress report will be organized around the five major aims i-v, 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 is used to control our fluorescence spectrometer and to digitize and store spectral data. The data can be analyzed and processed using the LABTECH NOTEBOOK software system which has also been purchased. Thus, our fluorescence spectrometer can now be used in this computer mode for fluorescence energy transfer experiments or for other fluorescence and phosphorescence spectroscopy work.

As an example of the latter, we have completed a study of phosphorescence in calf lens crystallins gamma-II, III, and IV during the past year. Measurements were made in our own laboratory at 77 K and in collaboration with Drs. Berger and Vanderkooi, University of Pennsylvania, at room temperature. These studies are aimed at revealing structural differences among the calf lens gamma crystallins in room temperature aqueous buffer solution. The intensity and lifetime of room

temperature phosphorescence are particularly sensitive to the permeability of the proteins to molecular oxygen. This work is in press [10], and a preprint is included in the appendix of this report.

For reasons given above (Sec. 1), we have not performed any tyrosine-to-tryptophan energy transfer studies on lens proteins during the past year, but the apparatus is ready if we should decide to reactivate this part of the project.

ii. Comprehensive studies of the local environments of the tryptophan residues in calf gamma crystallin subfractions gamma-II, -III, and -IV [3] and in the alpha and beta crystallin fractions [2], using the fluorescence quenching method, have already been completed. The main conclusion was 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. These conclusions agree with those from Chakrabarti's lab [11], based on other kinds of spectroscopic measurements. In addition, our efforts have stimulated two other labs to use the acrylamide quenching method to study lens protein structure [4,5,6].

In addition to the work on acrylamide quenching of tryptophan fluorescence in lens crystallins, which yielded structural information for these proteins, we have now completed a fundamental study of the physical mechanism of acrylamide quenching of tryptophan excited states in general. In this work we performed kinetic studies of acrylamide quenching of tryptophan photolysis and photoionization, and compared these results with the kinetics of fluorescence quenching in tryptophan. We found that fluorescence quenching was the most efficient of the three quenching processes, being about 3-4 times faster than quenching of photolysis or photoionization. This work is now in press [7]. The photoionization quenching work was done in collaboration with Prof. J. S. Huebner, University of North Florida, who came to Dr. Borkman's lab in Atlanta for three months in 1987. The relevance to this work to our project's overall goals is that acrylamide has now been shown to be an effective quencher of photochemistry and photoionization in tryptophan, and these processes have been implicated in lens photodamage mechanisms [12-14]. Hence, acrylamide could potentially serve as an anti-UV-cataract agent in the lens.

iii. We have done a number of photodamage experiments on whole lenses to date. The apparatus used for these studies allows 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 a LUMONICS excimer laser, purchased with funds from this grant. This laser can output radiation at wavelengths of 350, 337, and 308 nm, all of which are of particular interest

for lens studies. The apparatus used in these experiments is described in the preprint by Dillon, et al, which is included in the appendix of this report. Some typical light scattering and SDS-PAGE data are also shown there. UV exposed lenses display significant opacification and increased yellow-brown pigmentation, when irradiated at 308 nm, but show little change upon 350 nm irradiation. This is most likely due to the greater UV absorbance of the lens at 308 compared to 350 nm. The visible light transmission of a whole calf lens exposed to 308 nm excimer laser radiation for up to 50 minutes decreases substantially during the first 15-20 minutes of irradiation and then levels off at a constant value for the remaining irradiation period. These short irradiation times are very important since they reduce the amount of time that lenses must kept in vitro. Such experiments would not be possible without the excimer laser. SDS-PAGE analysis of proteins extracted from irradiated whole lenses show protein crosslinking.

iv. This topic is being very actively pursued and we will continue to do so in the coming year. A fluorescence detector for HPLC, has been purchased with funds from this grant and incorporated into the HPLC system. We have found appropriate digestion conditions for both trypsin and cyanogen bromide digestion of gamma-II crystallin, and have chromatographically resolved and collected some of the tryptophan-containing digestion peptides from UV irradiated gamma-II crystallin. These have been sequenced allowing us to assign chromatographic peaks to specific tryptic peptides. In this way we are able to monitor the photo-chemical loss of individual tryptophan residues, of known sequence number, in gamma-II crystallin.

v. We have completed a study of photolysis of the calf gamma crystallin subfractions gamma-II, III and IV, and this work is now in press and a preprint is in the appendix [Walker and Borkman]. 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 photosensitizers such as N-formylkynurenine (NFK) and riboflavin. We found that the gamma-IV fraction reacted most rapidly to produce increased visible light scattering, both with and without sensitizers. Sensitizers increased 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. The insoluble fraction was collected and found by SDS-PAGE to contain crosslinked protein aggregates, at 40,000 and 60,000 dalton and at very high molecular weight (did not enter the gels).

In another study, irradiated whole lenses were analyzed by Western blot, and it was established that the high molecular weight protein, produced by UV, contained gamma crystallin.

This work was done in collaboration with Drs. Dillon, Spector, and Roy at Columbia University. A manuscript has been submitted for publication based on this work and is included in the appendix.

3. HUMAN SUBJECTS

There are no changes in this aspect of the project. The only use of human subjects is that some experiments require proteins isolated from human lens specimens from Eye Bank donors.

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.

5. PUBLICATIONS

(Preprints included in the Appendix.)

M. L. Walker and R. F. Borkman, "Light Scattering and Photocrosslinking in the Calf Lens Crystallins Gamma-II, III, and IV", Exp. Eye Res. 48, 000 (1989).

J. W. Berger, J. M. Vanderkooi, D. H. Tallmadge, and R. F. Borkman, "Phosphorescence Measurements of Calf Gamma-II, III, and IV Crystallins at 77 and 293 K", Exp. Eye Res. 48, 000 (1989).

D. H. Tallmadge, J. S. Huebner, and R. F. Borkman, "Acrylamide Quenching of Tryptophan Photochemistry and Photophysics", Photochem. Photobiol. 48, 000 (1989).

J. Dillon, D. Roy, A. Spector, M. Walker, L. Hibbard, and R. Borkman, "UV Laser Photodamage to Whole Lenses", Exp. Eye Res. (submitted, 1988).

6. LITERATURE CITED:

1. Borkman, R. F. and S. R. Phillips (1985) "Tyrosine-to-Tryptophan Energy Transfer and the Structure of Calf Gamma-II Crystallin", Exp. Eye Res. 40, 819-826.

2. Phillips, S. R., L. Wilson, and R. Borkman (1986) "Acrylamide and Iodide Fluorescence Quenching as a Structural Probe of Tryptophan Microenvironment in Bovine Lens Crystallins", Curr. Eye Res. 5, 611-619.

3. Phillips, S. R. and R. F. Borkman (1988) "Fluorescence Quenching Studies of the Structures of Calf Gamma-II, III, and IV Crystallins", Curr. Eye Res. 7, 55-59.

4. Mandal, K. and B. Chakrabarti (1988) "Structure and Stability of Gamma Crystallins: Tryptophan, Tyrosine and Cysteine Accessibility", Biochemistry 27, 4564-4571.

5. Augusteyn, R.C., T. Putilina, and R. Seifert (1988) "Quenching of Tryptophan Fluorescence in Bovine Lens Proteins by Acrylamide and Iodide", Curr. Eye Res. 7, 237-245.

6. Augusteyn, R.C., M. Ferraro, and J. Thomson (1988) "Probing the Microenvironments of Tryptophan Residues in the Monomeric Crystallins of the Bovine Lens", Curr. Eye Res. 7, 823-828.

7. Tallmadge, D. H., J. S. Huebner, and R. F. Borkman (1989) "Acrylamide Quenching of Tryptophan Photochemistry and Photophysics", Photochem. Photobiol. 48, 000-000 (in press).

8. Walker, M. L. and R. F. Borkman (1989) "Light Scattering and Photocrosslinking in the Calf Lens Crystallins Gamma-II, III, and IV", Exp. Eye Res. 48, 000 (in press)

9. Dillon, J., D. Roy, A. Spector, M. Walker, L. Hibbard, and R. Borkman (1989) "UV Laser Photodamage to Whole Lenses", Exp. Eye Res. (submitted, 1988).

10. Berger, J., J. Vanderkooi, D. Tallmadge, and R. Borkman (1989) "Phosphorescence Measurements of Calf Gamma-II, III, and IV Crystallins at 77 and 293 K", Exp. Eye Res. 48, 000-000.

11. Mandal, K., S. Bose, B. Chakrabarti, and R. Siezen (1985) "Structure and Stability of Gamma Crystallins. I. Spectroscopic Evaluation of Secondary and Tertiary Structure in Solution", Biochim. Biophys. Acta 832, 156-164.

12. Dillon, J. and A. Spector (1982) "A Comparison of Aerobic and Anaerobic Photolysis of Lens Protein", Exp. Eye Res. 31, 591-597.

13. Zigman, S. (1976) "Tryptophan Excited States in the Lens", Doc. Ophthalmol. 8, 267-274.

14. Kurzel, R.B., M. Wolbarsht, B.S. Yamanashi, G.W. Staton, and R.F. Borkman (1973) "Tryptophan Excited States and Cataracts in the Human Lens", NATURE (London) 241, 132-133.

APPENDIX

Copies of four preprints listed in the body of the report, and completed during the past project year:

M. L. Walker and R. F. Borkman, "Light Scattering and Photocrosslinking in the Calf Lens Crystallins Gamma-II, III, and IV", Exp. Eye Res. 48, 000 (1989).

J. W. Berger, J. M. Vanderkooi, D. H. Tallmadge, and R. F. Borkman, "Phosphorescence Measurements of Calf Gamma-II, III, and IV Crystallins at 77 and 293 K", Exp. Eye Res. 48, 000 (1989).

D. H. Tallmadge, J. S. Huebner, and R. F. Borkman, "Acrylamide Quenching of Tryptophan Photochemistry and Photophysics", Photochem. Photobiol. 48, 000 (1989).

J. Dillon, D. Roy, A. Spector, M. Walker, L. Hibbard, and R. Borkman, "UV Laser Photodamage to Whole Lenses", Exp. Eye Res. (submitted, 1988).