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ENERGY TRANSFER MECHANISMS IN PHOTOBIOLOGICAL REACTIONS

Progress Report
for Period 1 August 1975 - 30 April 1976

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TECHNICAL PROGRESS REPORT

Introduction

This report will be brief since much of the research performed is described in the reprints and abstracts of papers presented at meetings as listed in the Appendix. At appropriate places these will be referred to by a letter corresponding to the Appendix section together with the number of the item in the section. Copies of these reprints, manuscripts, etc. have been submitted to ERDA.

The principal investigator spent half-time on this research project during the autumn quarter of 1975 and the winter quarter of 1976; he will do likewise during the spring quarter of 1976 and will devote full time for two months to the project during the summer (non-teaching) quarter of 1976.

Abstract of Research Accomplishments

As in our earlier work on the sensitized photooxidation of substituted phenylalanines, ring substituents were found to have a marked effect on the photooxidation of uracil. This compound was selected as a typical pyrimidine for detailed studies of the mechanism of photooxidation of molecules of biological importance. The photooxidation of a series of 5-substituted uracils as sensitized by eosin Y, ruthenium bipyridyl, 3-methyl-lumiflavin and methylene blue was studied using oxygen electrode and flash photolysis techniques. Different substituents had very different effects on the rates of photooxidation with a given dye, and some substituents could shift the mechanism from a singlet oxygen pathway to an electron abstraction pathway (or vice versa). A study of the effects of porphyrin structure in relation to photosensitizing characteristics was completed. An examination of the localized selective photooxidation of amino acids in the vicinity of the heme binding site of horseradish peroxidase was made using specifically-bound protoporphyrin IX and other porphyrins as sensitizers. Preliminary studies were carried out on the use of sensitized photooxidation as a tool to study the contribution of histidine-containing crosslinks to the mechanical-thermal properties of mammalian tendon as well as the role of membrane properties in determining the rate and direction of swimming of certain microorganisms.

Summary of Other Accomplishments

1. Publications. As listed in the Appendix, three papers were published during the present reporting period. Another manuscript, as listed, is in press and several others are in preparation.

2. Meetings. Four papers were presented at meetings during the reporting period as listed in Appendix B. Three more, including two invited talks, will be given at national and international meetings during the coming summer (Appendix C).

3. Educational activities. Two graduate students are presently carrying out their Ph.D. research under the project. Historically, on this project, graduate students have been authors or co-authors of most of the published papers and have presented many of the papers at meetings. Two of the papers listed in

Appendix B (Nos. 2 and 4) were presented by graduate students. Three undergraduate biology students have worked as technicians on the project, and three biology students have carried out small senior research projects on the program during the present period.

4. Miscellaneous activities. I served as immediate Past President of the American Society for Photobiology during the reporting period and also edited a Newsletter which the Society publishes. I was invited to help organize and to chair a symposium entitled "Photosensitized reactions of nucleic acids and proteins" which will be given as part of the Seventh International Congress on Photobiology to be held in Rome this coming summer.

Summary of Research Activities

Research activities for the project year to date are reported below under the same general headings and subdivisions used in the most recent Proposed Technical Program dated 1 May 1975. Not all of the areas of research proposed in that document have been worked on during this report period since the proposal was intended to encompass a three year period.

A. Studies on the Mechanisms of Photodynamic Reactions

1. Effect of substituent groups on the mechanism of the photooxidation of substrates.

a. The sensitized photooxidation of phenylalanine derivatives. We held up our work on this topic (as detailed in the last Progress Report) in order to repeat our measurements of quantum yields for the photooxidation of the different derivatives using an improved oxygen electrode apparatus which we constructed. In addition we extended our studies on these reactions as carried out in the presence of azide (which quenches singlet oxygen) and in heavy water (which lengthens the lifetime of singlet oxygen by a factor of ten). These additional measurements have been very useful in further defining the mechanisms involved. The revised paper on these studies as carried out with eosin Y as sensitizer is now almost complete; the paper concerning flavin sensitizers will be completed this summer.

b. The sensitized photooxidation of substituted uracils. This area has been one of our major activities this year. The work has proceeded well, and the first presentation of the results will be given at the Rome photobiology congress in September (A-3). In brief, we have examined the effects of the following substituents in the 5-position on the quantum yield of photooxidation of uracil: H, F, Cl, Br, I, CH_3, OH, COOH, NO_3 and NH_2. Measurements have been made as a function of pH, oxygen concentration, light intensity, etc. with four sensitizers: eosin Y, 3-methyl lumiflavin, ruthenium bipyridyl and methylene blue. Effects of heavy water and azide are being examined, and we have just started flash photolysis measurements to further establish the reaction pathways. This area of research will be continued for another year or more.

2. Mechanisms of the sensitized photooxidation of methionine and other amino acids.

Research in this area during the present contract year was confined to studies with porphyrins and with ruthenium bipyridyl as sensitizers; the results are described in the next two sections of this report (3. and 4.). In addition,

some experiments on the sensitized photooxidation of amino acids with bound sensitizers were carried out as described in section B.2. of this report. The proposed comparative study on amino acid photooxidation as sensitized by eosin Y and 3-methyl lumiflavin has not been pursued this year but will be continued in the coming contract year.

3. Mechanisms of porphyrin-sensitized photooxidation reactions.

a. Porphyrin structure and photosensitizing efficiency. This work has been largely completed. It has been carried out jointly with Dr. Giulio Jori of the University of Padova, Italy, under the U.S.-Italy Cooperative Science Programs and was described in some detail in the last progress report. In the present contract year we have largely completed the flash photolysis aspects of the work, concentrating on the photochemical behavior of metal-free and metal-containing hematoporphyrin as a type-compound. Two long-lived excited states of hematoporphyrin are observed on flashing in acetic acid solution. One is the triplet (lifetime 5.3×10^{-4} sec) while the other is a semi-reduced form (lifetime 2.8×10^{-3} sec). Oxygen quenches the triplet state with high efficiency in a reaction giving a good yield of singlet oxygen. The amino acid methionine does not quench the triplet, indicating that the rather efficient photooxidation of methionine with hematoporphyrin must proceed via a singlet oxygen mechanism. A paper on this work is in preparation.

b. Deuteroporphyrin-sensitized photooxidation reactions. We continued our studies of deuteroporphyrin sensitized reactions (B-2). Quantum yields of 0.02-0.27 were found for the sensitized photooxidation of methionine, histidine and tryptophan in aqueous solution buffered at pH 12 with Zn^{++} deuteroporphyrin IX as sensitizer. Quantum yields were increased by a factor of 3-5 in heavy water. This suggests that the reactions were mediated by singlet oxygen, which is consistent with our flash photolysis studies on this system.

c. Sensitized photooxidation studies on horseradish peroxidase. This work was described in detail in the last progress report. One area has been completed (the porphyrin-sensitized photooxidation of horseradish peroxidase) and published (A-3). Since reprints of this paper have been submitted to ERDA, no further description of the results will be given here.

4. Complexed metal ions as photodynamic sensitizers.

This work was carried out primarily with tris(2,2'-bipyridine)ruthenium II chloride (usually termed ruthenium bipyridyl) as sensitizer. The charge-transfer triplet state of this compound is efficiently quenched by molecular oxygen in a reaction which gives singlet oxygen in a good yield. Further, the compound has a large extinction coefficient and is both photochemically stable and water soluble, which suggested that it would be a useful photodynamic sensitizer for biological substrates. Ruthenium bipyridyl proved to be an efficient sensitizer for the photooxidation of the five susceptible amino acids: cysteine, histidine, methionine, tryptophan and tyrosine. Quantum yields for photooxidation were measured as a function of light intensity, amino acid concentration, sensitizer concentration, oxygen concentration and pH (aqueous solution, sodium phosphate buffer). The sensitizing efficiency of ruthenium bipyridyl appeared to be constant over the pH range 2-12. This permitted an unambiguous determination of the effects of pH on the sensitivity of amino acids *per se* to photooxidation; each amino acid shows a characteristic pH-rate dependence. Photo-

oxidation rates were strongly enhanced in heavy water and inhibited by sodium azide suggesting that photooxidation was mediated by singlet oxygen. Superoxide dismutase had no effect. Ruthenium bipyridyl was also an efficient sensitizer for the photodynamic inactivation of the enzyme lysozyme; again the rate of photoinactivation was sharply increased in heavy water. Work is continuing in this area.

5. Photodynamic studies with proteins.

As described in the last progress report, we have been carrying out a detailed study using flash photolysis techniques, etc. of the mechanisms involved in the chemical inhibition of the eosin-sensitized photooxidation of trypsin. Since this work has now been published (A-1) and reprints have been submitted to ERDA, it will not be discussed further in this report.

B. Selective Photodynamic Treatment as a Tool in Studies of Selected Biological Systems

This represents a new aspect of research in our program as proposed and documented in detail in the last renewal proposal dated 1 May 1975. Preliminary studies were initiated in both subdivisions of this area as proposed.

1. Selective effects of photodynamic treatment on the mechanical-thermal properties of mammalian tendon (collagen).

Preliminary studies on the photodynamic destruction of amino acid residues in rat tail tendon were carried out. As predicted in our proposal, the results indicate that with certain sensitizers and under certain conditions, histidine residues in the tendon can be selectively destroyed. We are presently assembling an apparatus (as provided by University funds) for making measurements on the mechanical-thermal properties of tendon. This area will be one of our major thrusts during the coming contract year.

2. Artificial sensitization of photoresponses in motile microorganisms.

Preliminary studies were carried out in this area, which is also new for the present contract year. As described in the proposal, this research is concerned with attempts to sensitize microorganisms to behavioral control by light (velocity and direction of swimming) by the use of membrane-incorporated photosensitizers. We have built up a microscope system including a video camera, a videotape recorder and videomonitor (using funds provided by the University of Utah) for recording the swimming responses of photosensitized microorganisms to light and have carried out very preliminary studies with paramecia sensitized with eosin Y and rose bengal. These initial studies are encouraging, and this area will represent another of our major thrusts during the coming contract year.

Sensitizing dyes bound in membranes would be expected to show a somewhat different photochemical behavior from the same dyes free in solution. Because of this we carried out a preliminary examination of the sensitized photooxidation of amino acids using sensitizing dyes bound to solid particles (A-6). The first experiments were performed with the water-soluble dyes methylene blue and

eosin which, when free in solution, are efficient sensitizers for the photooxidation of amino acids. Both dyes are ionic (methylene blue$^+$ and eosin$^-$) over a wide pH range. We found that methylene blue and eosin are bound strongly and practically irreversibly to strong cationic (Bio-Rad AG50W-X12) and anionic (Bio-Rad AG1-X10) exchange resins respectively and are efficient sensitizers in the ionically bound form. Up to 60 μmoles of dye could be bound per gram of resin without any dye being measurable in the reaction system before or after illumination. We have compared the kinetics for the photooxidation of amino acids with free vs bound sensitizer in order to determine whether ionic binding of sensitizer on a solid styrene-divinylbenzene polymer lattice alters the mechanism of the photooxidation reaction. Photooxidation rates for histidine, methionine, tyrosine and tryptophan with bound dyes were approximately 1/3 to 1/2 those observed with free dyes under similar conditions. Kinetic differences were found which suggest that the mechanisms of photooxidation are different with free vs bound sensitizers. These include the absence or reduction of the D_2O effect; the continued dependence of photooxidation rate on substrate concentration at relatively high substrate concentrations; the stronger effect of free radical inhibitors; and a decreased effect of singlet oxygen scavengers on the rate with bound sensitizers. Irreversibly-bound sensitizers are advantageous for certain studies and it is likely that many sensitized photooxidation reactions in complex biological systems such as cells and cell membranes would occur via sensitizer bound ionically to charged biopolymers.

APPENDIX A - Published Papers and Abstracts, Papers in Press, Manuscripts, and Reports.

1. "Chemical inhibition of the eosin-sensitized photooxidation of trypsin," by F. Rizzuto and J.D. Spikes. Radiation and Environ. Biophysics 12: 217-232 (1975). (COO-875-154).

2. "Porphyrins and related compounds as photodynamic sensitizers," by J.D. Spikes. Annals N.Y. Acad. Sci. 244:496-508 (1975). (COO-875-160)

3. "The porphyrin-sensitized photooxidation of horseradish apoperoxidase," by Yeou-Jan Kang and J.D. Spikes. Arch. Biochem. Biophys. 172: 565-573 (1976). (COO-875-162)

4. "Photosensitization," by J.D. Spikes. In press, in The Science of Photobiology (K.C. Smith, editor), Plenum Press, New York (1976). (COO-875-168).

5. "Tris(2,2'-bipyridine)ruthenium II chloride as a sensitizer for the photooxidation of amino acids and proteins," by J.D. Spikes. Program and Abstracts of the 4th Annual Meeting of the American Society for Photobiology, pp. 79-80, Denver, 16-20 February 1976. (COO-875-174).

6. "Sensitized photooxidation of amino acids by methylene blue (Mb) and eosin Y (EoY) bound to strong ion exchange resins," by R. Straight and J.D. Spikes. Program and Abstracts of the 4th Annual Meeting of the American Society for Photobiology, p. 80, Denver, 16-20 February 1976. (COO-875-175).

7. Annual ERDA Technical Progress Report, by J.D. Spikes, 1 May 1976. (COO-875-176).

APPENDIX B - Papers Presented at Meetings.

1. "Ruthenium coordination compounds as photosensitizers for biological systems," by J.D. Spikes. Presented at the Gordon Conference on Organic Photochemistry, Tilton School, New Hampshire, 1 August 1975. (COO-875-170).

2. "Quantum yield measurements of the porphyrin-sensitized photooxidation of amino acids in aqueous solution," by G.D. Coker, J.D. Spikes and B.F. Burnham. Presented at the meetings of the Utah Academy of Sciences, College of Southern Utah, Cedar City, Utah, 24 October 1975. (COO-875-173).

3. "Tris(2,2'-bipyridine)ruthenium II chloride as a sensitizer for the photooxidation of amino acids and proteins," by J.D. Spikes. Presented at the annual meeting of the American Society for Photobiology, Denver, 16-20 February 1976. (COO-875-174).

4. "Sensitized photooxidation of amino acids by methylene blue (Mb) and eosin Y (EoY) bound to strong ion exchange resins," by R. Straight and J.D. Spikes. Presented at the annual meeting of the American Society for Photobiology, Denver, 16-20 February 1976. (COO-875-174).

APPENDIX C - Papers to be Presented at Meetings.

1. "Fundamentals of porphyrin photochemistry," by J.D. Spikes. To be presented (by invitation) at the Gordon Conference on Chemistry and Biology of Pyrroles, Brewster Academy, Wolfsborough, New Hampshire, 2-6 August 1976. (COO-875-177).

2. Chairman's introduction to a symposium "Photosensitized reactions of nucleic acids and proteins," by J.D. Spikes. To be presented (by invitation) at the Seventh International Congress on Photobiology, Rome, Italy, 29 August - 3 September 1976. (COO-875-178).

3. "Substituent effects on the sensitized photooxidation of uracil," by J.D. Spikes. To be presented at the Seventh International Congress on Photobiology, Rome, Italy, 29 August - 3 September 1976. (COO-875-179).

Copies of the above items have been submitted to ERDA.