ENERGY TRANSFER MECHANISMS IN
PHOTOBIOLOGICAL REACTIONS

Progress Report
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MASTER

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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 project during September 1976; half time during the 9-month 1976-77 academic year; and is spending full time on it for three months during the 1977 summer (non-teaching) quarter.

To our knowledge, we have complied fully with all contract requirements.

Abstract of Research Accomplishments

During this second year (of the 3-year cycle) we have continued the work of the first year on the relationship between the chemical structure of biomolecules and the kinetics and mechanisms of their sensitized photooxidation. A major paper (A-3)* was published on the role of substituent groups on the photooxidation of tyrosine and related compounds, and two papers were presented at meetings (A-3, A-4) on the effects of substituents in the 5-position on the mechanism of the sensitized photooxidation of uracils. In addition, a study of the relationship between the structure and photosensitizing efficiencies of porphyrins was completed and published (A-2). Finally, a study of the effects of the spin-state of the iron in the heme moiety of horseradish peroxidase on the sensitivity of the enzyme to photodynamic treatment was completed and published (A-1).

Also, in this second year, work has gotten well under way on the effects of photodynamic treatment on mammalian tendon. By using different sensitizers, solvents and reaction conditions, we have been able to get a reasonably selective photooxidation of the different susceptible amino acid residues in tendon. Of greater interest, we have shown that the changes in properties of photodynamically-treated tendons result from the formation of cross-links in the protein rather than from the photochemical alteration of amino acid side chains as such.

Summary of Other Accomplishments

1. Publications. As listed in Appendix A, three papers were published on research carried out under this contract during the present reporting period. Three other manuscripts (including a lengthy critical review of the use of photochemical techniques in mapping the three-dimensional structures of proteins) are in press, while another has been submitted for publication.

* Refers to items as listed in the different sections of the Appendix.

2. Meetings. Four papers were presented at national and international meetings as listed in Appendix B. Two were presented by invitation, one at the Gordon Conference on the Chemistry and Biology of Pyrroles and one at the Seventh International Congress on Photobiology in Rome.

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. Three undergraduate biology students have worked as technicians on the project, and four biology students carried out small senior research projects on the program during the present period.

4. Miscellaneous activities. The principal investigator was invited to chair a symposium entitled "Photosensitized reactions of nucleic acids and proteins" held as part of the Seventh International Congress on Photobiology in Rome. He is also a past president of the American Society for Photobiology.

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. This work was completed and published as a major paper (A-3). Since reprints of this paper have been submitted to ERDA, the results will not be described here.

b. The sensitized photooxidation of substituted uracils. This work is developing well and will be completed during the coming contract year. Two papers in this area were presented at national and international meetings (B-3, B-4). As is well-known, different nucleic acid bases show markedly different rates of photooxidation on illumination in the presence of photodynamic sensitizers. This paper explores the effects of various substituents in the 5-position of uracil on the kinetics and mechanisms of photooxidation. Rates of oxidation were measured in terms of oxygen uptake using an oxygen electrode system. With eosin^Y as sensitizer (and also with methylene blue and ruthenium bipyridyl), uracil is photooxidized only very slowly ($\phi = 0.0019$) at pH 8; the rate increases progressively with increasing pH ($\phi = 0.023$ at pH 11.6). At pH 8 and above, halogen substituents in the 5-position increase the rate of photooxidation in the order F ($\phi = 0.0088$ at pH 8, 0.089 at pH 11.6) > Cl > Br ~ I. A 5-methyl substituent also increases the rate. An amino group in the 5-position markedly increases the rate of photooxidation ($\phi = 0.13$ at pH 8 and 0.78 at pH 11.6), as did a hydroxy group ($\phi = 0.39$ at pH 8 and 0.81 at pH 11.6). Azide, which quenches singlet oxygen, had only a small effect on the rate of the eosin-sensitized photooxidation of uracil at pH 8 (50% inhibition at $2 \times 10^{-1} M$), but was strongly inhibitory at pH 11 (50% inhibition at $1.1 \times 10^{-3} M$). In D_2O , the rate was increased two-fold at pH 8 and over five-fold at pH 11. At both pH 8 and pH 11, the photooxidation

rates of 5-hydroxyuracil and 5-aminouracil were only mildly inhibited by azide (50% inhibition at $> 1 \times 10^{-1} \text{M}$). Flash photolysis studies showed that uracil and halogen-substituted uracils did not appreciably quench the eosin triplet whereas 5-hydroxyuracil and 5-aminouracil quenched it strongly. In contrast to the eosin results, uracil was photooxidized very rapidly with 3-methyl lumiflavin as sensitizer ($\phi = 0.22$ at pH 8 and 0.72 at pH 11.6). At pH values above 8, halogen substituents decreased the rate of photooxidation in the order $\text{Br} \sim \text{I} > \text{Cl} > \text{F}$. 5-Hydroxyuracil and 5-aminouracil were photooxidized at approximately half the rate of uracil, while 5-methyluracil was photooxidized even more slowly. The flavin-sensitized photooxidation of uracil was very sensitive to azide inhibition at pH 8 (50% inhibition at $1.5 \times 10^{-5} \text{M}$) and somewhat sensitive at pH 11 (50% inhibition at $3.5 \times 10^{-4} \text{M}$).

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

We initiated work in a new aspect of this area; i.e., on the role of superoxide radical in the sensitized photooxidation of amino acids. The superoxide radical is generated on the aerobic illumination of a number of photodynamic sensitizers; however, the possible role of this species in the sensitized photooxidation of biological substrates is not known unequivocally. In the present work, the effects of bovine erythrocyte superoxide dismutase (SOD) on the rate of the 3-methyl lumiflavin-sensitized photooxidation of cysteine, histidine, methionine, tryptophan, tyrosine, dihydroxyphenylalanine, epinephrine and 4-methoxyphenylalanine were surveyed at pH 6.5 and 9. The effects of catalase and of catalase + SOD during illumination were also examined. Quantum yields of photooxidation were determined by measuring oxygen uptake with a recording oxygen electrode system; hydrogen peroxide production was measured at the end of the illumination period using catalase. On the basis on the preliminary experiments, tyrosine and epinephrine were selected for more detailed kinetic studies. The rate of oxygen uptake during the photooxidation of tyrosine was increased over two-fold by SOD. The effect showed a fairly sharp maximum at pH ~ 6.5 and increased with increasing SOD concentration up to $\sim 0.05 \text{ mg/ml}$. Boiled SOD had no effect. There was a small increase in the yield of hydrogen peroxide during photooxidation in the presence of SOD. Addition of catalase at the start of an experiment decreased the apparent rate of oxygen consumption on illumination, presumably as a result of the dismutation of the hydrogen peroxide produced photochemically. In contrast to the results with tyrosine, SOD had little effect on the rate of oxygen consumption during the flavin-sensitized photooxidation of epinephrine at pH 6.5, and significantly decreased the rate of oxygen uptake at pH 8.1. Free radical scavengers such as ethanol did not alter these SOD effects on photooxidation rates. This work is continuing. A paper on this will be given at an international meeting in August (C-1).

3. Mechanisms of porphyrin-sensitized photooxidation reactions.

a. Porphyrin structure and photosensitizing efficiency. This area of work was completed and a paper published (A-2). Since reprints of this publication have been submitted to ERDA, this work will not be described further here.

b. Sensitized photooxidation studies on horseradish peroxidase. Another area of this work (an examination of the sensitized photooxidation of horseradish peroxidase in which the heme iron has been converted to a low spin state) has been

completed and published (A-1). Again, since reprints have been sent to ERDA, no further discussion will be given here. This work is continuing; some aspects will be presented in an invited symposium paper in August (C-2).

4. Complexed metal ions as photodynamic sensitizers.

This work was suspended temporarily to permit concentration on the possible role of superoxide anion in photodynamic reactions as described above. It will be taken up again during the coming year.

5. Photodynamic studies with proteins.

Work in this area on the eosin-sensitized photooxidation of trypsin was completed and published as described in the last technical progress 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. Because of manpower limitations, we concentrated this year on part 1 of this research area.

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

This area represented one of our major activities during the contract year. A number of dyes were screened for their ability to sensitize the photooxidation of collagen. A group of dyes representing different molecular structures, charge and solubility properties, was selected for further study. Studies were carried out with saline and with ethanol as solvents and with pre-stained tendons. Using different dyes and reaction conditions (in particular, solvent and pH) it was possible to obtain somewhat selective photooxidation of the three types of sensitive residues in tendon (histidine, methionine and tyrosine). Only those dyes which sensitized the photodynamic destruction of amino acids were able to alter the mechanical and thermal shrinkage properties of tendons on illumination. However, the resulting changes in tendon properties (by comparison with chemically cross-linked tendons) indicated that the effects of photodynamic treatment must result primarily from the photochemical formation of cross-links rather than from the photodynamic breakage of pre-existing histidine-containing cross links. This interpretation was confirmed by studies of the solubility of photodynamically-treated tendon in 0.1M acetic acid. Native rat tail tendon is almost completely soluble in this solvent, while tendons treated with known chemical cross-linking agents show a progressive decrease in solubility with increasing cross linking. In our experiments the solubility of tendons in 0.1M acetic acid decreased progressively with increasing time of photodynamic treatment. This work is continuing as described in the accompanying renewal proposal.

2. Artificial sensitization of photoresponses in motile microorganisms.

Due to manpower limitations, only a small amount of research was carried out in this area during the contract year.

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

1. "Sensitized photooxidation of low spin horseradish peroxidase," by Yeou-Jan Kang and J. D. Spikes. *Biochem. Biophys. Research Commun.* 74: 1160-1167 (1977).
2. "The effect of chemical structure on the photosensitizing efficiencies of porphyrins," by G. Cauzzo, G. Gennari, G. Jori and J. D. Spikes. *Photochem. Photobiol.* 25: 389-395 (1977).
3. "The eosin-sensitized photooxidation of substituted phenylalanines and tyrosines," by F. Rizzuto and J. D. Spikes. *Photochem. Photobiol.* 25: 465-476 (1977).
4. "Photosensitization," by J. D. Spikes. *In the science of photobiology* (K. C. Smith, ed.) Plenum Press, New York (in press, 1977).
5. "Photosensitization in biological systems," by J. D. Spikes. *In research in Photobiology* (A. Castellani, ed.) Plenum Press, New York (in press, 1977).
6. "Mapping the three-dimensional structure of proteins by photochemical techniques," by G. Jori and J. D. Spikes. *In Photochem. Photobiol. Revs.*, Vol. 3 (in press, 1977).
7. "Sensitized photooxidation of amino acids: effects in the reactivity of their primary amino group with fluorescamine and o-phthalaldehyde," by R. Straight and J. D. Spikes. Submitted to *Biochem. Biophys. Research Commun.* (1977).
8. "Effects of substituents in the 5-position on the photooxidation of uracil as sensitized by eosin Y and 3-methyl lumiflavin," by J. D. Spikes. Page 522, in *Book of Abstracts, Seventh International Congress on Photobiology, Rome, 29 August - 3 September, 1977* (Abstract).
9. "Kinetics of the eosin Y sensitized photooxidation of substituted uracils," by J. D. Spikes. Page 47, *Book of Abstracts, 5th Annual Meeting, American Society for Photobiology, San Juan, Puerto Rico, 11-15 May 1977* (Abstract).
10. "Annual ERDA Progress Report," by J. D. Spikes, 31 July 1977 (COO-875-178).

APPENDIX B - Papers Presented at Meetings.

1. "Fundamentals of porphyrin photochemistry," by J. D. Spikes. Presented by invitation at the Gordon Conference on Chemistry and Biology of Pyrroles, Brewster Academy, Wolfborough, New Hampshire, 2-6 August 1976.
2. "Photosensitization in biological systems," by J. D. Spikes. Presented by invitation as the introductory lecture on a symposium entitled Photosensitized Reactions of Nucleic Acids and Proteins, held as part of the Seventh International Congress on Photobiology, Rome, Italy, 29 August - 3 September 1976.

3. "Effects of substituents in the 5-position on the photooxidation of uracil as sensitized by eosin Y and 3-methyl lumiflavin," by J. D. Spikes. Presented at the Seventh International Congress on Photobiology, Rome, Italy, 29 August - 3 September 1976.
4. "Kinetics of the eosin Y sensitized photooxidation of substituted uracils," by J. D. Spikes. Presented at the 5th Annual Meeting of the American Society for Photobiology, San Juan, Puerto Rico, 11-15 May 1977.

APPENDIX C - Papers to be Presented at Meetings

1. "The role of superoxide radical in the flavin-sensitized photooxidation of amino acids," by J. D. Spikes. To be presented at the International Congress on Singlet Oxygen and Related Species, Pinawa, Manitoba, Canada, 21 - 26 August 1977.
2. "Porphyrins as photodynamic sensitizers," by J. D. Spikes. To be presented by invitation as part of a symposium entitled Biological Photosensitization at the meetings of the American Chemical Society, Chicago, Illinois, 28 August - 2 September 1977.
3. "Fundamental aspects of photosensitization and photodynamic action," by J. D. Spikes. To be presented by invitation as the introductory talk on a symposium entitled Fundamental Photobiology which will be held as part of the Fourteenth International Congress on Radiology, Rio De Janeiro, Brazil, 23 - 29 October 1977.
4. "Primary photochemical events and their relation to photobiology," by J. D. Spikes. To be presented by invitation at the Institute of Biophysics, Universidade Federal do Rio De Janeiro, Brazil, on 25 October 1977.