DOE/ER13631--72
Data

DOE/ER13631

PHOTOCHEMICAL ENERGY CONVERSION BY MEMBRANE-BOUND PHOTOREDOX
SYSTEMS

FINAL REPORT

June 16, 1994

Grant #: DE-FG02-86ER13631

Principal Investigator: Gordon Tollin
Department of Biochemistry
University of Arizona
Tucson, AZ 85721

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

MASTER

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

mt

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

SUMMARY OF ACHIEVEMENTS

This project has received DOE support since July 1, 1978. During this period, 40 papers have been published dealing predominantly with chlorophyll-photosensitized electron transfer reactions in a variety of media (solutions, polymer films, lipid bilayer membranes). The overall theme of this work has been to develop mechanistic strategies for photochemical energy storage via chlorophyll, using the green plant photosynthetic system as a paradigm for designing in vitro systems. We decided to use chlorophyll largely because it has been developed by biological evolution as the transducing molecule for photosynthesis, and thus has many favorable properties. At the start of this project, we realized that the most likely medium with which to achieve success in this effort was the lipid bilayer, which also serves as the matrix for photochemical energy conversion in vivo. One of the useful properties of chlorophyll in this context is its ability to spontaneously orient itself in a lipid bilayer with its chlorin ring favorably disposed with respect to the membrane surface and the aqueous medium. Thus, although we continued during the first phases of the project to explore homogeneous solution systems (1,3,4; numbers refer to publication list below), we quickly turned entirely to microheterogeneous lipid vesicle suspensions as the medium of choice (2,5,6). This type of system allows ready application of time-resolved optical spectroscopy to follow the course of light-induced electron transfer processes, a methodology which has been applied with considerable success in elucidating the

events occurring during natural photosynthesis. Using quinones as electron acceptors from triplet state chlorophyll, we were able to show (5) that both the yields and the lifetimes of electron transfer products were markedly improved in the vesicle systems over what we could achieve in homogeneous solutions, as a direct consequence of compartmentalization of the reaction products, i.e. the oxidized chlorophyll remains membrane-bound whereas the reduced quinone is expelled from the membrane into the aqueous phase. In subsequent studies, we learned to favorably manipulate this compartmentalization by controlling the electrical charge on the membrane surface (9,11,13,21,22,24), by controlling the solubility properties of the acceptors (14), by varying the lipid composition (12), by using mediators to create a concentration gradient to carry electrons from within the bilayer to the aqueous medium (25,26,27), and by incorporating secondary electron acceptors into the aqueous phase (15).

An important development during this period was our demonstration (7,10) that chlorophyll could photosensitize vectorial electron transfer across the lipid bilayer from a donor located in the inner aqueous compartment to an acceptor located in the outer aqueous phase. This permitted the physical separation of the redox products, based upon an initial asymmetry imposed on the system by the solubility and transport properties of the materials. We have characterized the mechanism of this process and have used it in our more recent work to develop systems which more closely mimic the natural ones (see below). During the course of these

studies, we discovered (8,18) that the lipid vesicles displayed a remarkable inside-outside surface asymmetry, which could be manipulated to improve the properties of the vectorial transport systems.

Much of our work over the past 7 years has focussed on the use of redox proteins as electron acceptors and donors in chlorophyll-based lipid vesicle systems. These studies have been based upon our initial demonstrations that cytochrome c (16) and plastocyanin (17) could effectively function in this way if one used a membrane surface charge which was opposite in sign to that of the protein surface. Again, our decision to use redox proteins was based upon the fact that biological evolution has proceeded in this direction. We were particularly excited by our discovery of the feasibility of this approach, inasmuch as it allowed us to more closely mimic the biological systems in: (i) an in vitro multi-component construct which included an integral membrane redox protein (cytochrome c oxidase), as well as soluble redox proteins and small molecule mediators (27), and (ii) in a vectorial electron transport system which included reduced cytochrome in the inner aqueous compartment of the vesicle and oxidized ferredoxin in the outer phase (29,30). It is important to point out that this latter system closely mimics a key event in natural photosynthesis, and results in the permanent storage of approximately 0.7 V across the lipid bilayer in the oxidized and reduced products, with an overall quantum efficiency of approximately 3%. We consider this to be an outstanding achievement for an in vitro system. In further efforts to develop

biomimetic energy storage strategies, we were able to construct a vesicle system which utilizes light energy absorbed by chlorophyll to create a pH gradient across a membrane (33), and to improve the yield of vectorial electron transport from cytochrome to ferredoxin by incorporating a second (flavin based) photochemical system into the vesicle (36,37). Both of these achievements mimic events which occur during natural photosynthesis.

In the final two years of the project, we focussed our efforts on developing a lipid-membrane coated electrode system which would allow facile direct electron transfer processes involving redox proteins (31,32,34,35), something which has proven to be very difficult to achieve using conventional metal electrodes. This development has been quite successful, and we have exploited it with both soluble redox proteins using electrostatic binding of the protein to the membrane surface, as well as with integral membrane redox proteins such as cytochrome oxidase which can be incorporated into the interior of the bilayer (38). This has allowed us to achieve the direct conversion of light into electricity with reasonable efficiency in a chlorophyll-based redox protein electrode system (40).

In addition to the research publications noted above, we have written three overview articles summarizing various aspects of this work. These are cited in references 6, 28, and 37.

In summary, during the course of this project we have developed a wide range of strategies to couple chlorophyll photoexcitation and subsequent electron transfer events into the

storage of chemical energy. Our emphasis in this work has been to explore the basic principles upon which such systems can be made to operate, rather than to attempt to develop practical devices. We feel confident that the information that we have produced can be utilized in the future to improve the technology of solar energy conversion, as well as to develop new technologies only dreamed of by today's scientists and engineers.

The work described herein has been carried out in collaboration with a large number of talented postdoctoral students, whose names appear on the publications cited below. One graduate student (John Hurley) has received his Ph.D. degree based on this work, and one student (Glen Cheddar) received an M.S. degree. One undergraduate student (D. M. Moezzi) also participated in the project.

DOE-SPONSORED PUBLICATIONS

- 1- G. Cheddar, F. Castelli and G. Tollin, "Chlorophyll Photochemistry in Condensed Media I. Triplet Quenching and Electron Transfer to Quinone in Cellulose Acetate Films." Photochem. Photobiol. 32, 71 (1980).
- 2- J. Hurley, F. Castelli and G. Tollin, "Chlorophyll Photochemistry in Condensed Media II. Triplet Quenching and Electron Transfer to Quinone in Liposomes," Photochem. Photobiol. 32, 79 (1980).
- 3- G. Cheddar and G. Tollin, "Laser Photolysis and EPR Studies of Quinone Reduction by Chlorophyll Triplet in Various Organic Solvents," Photobiochem. Photobiophys. 1, 235 (1980).
- 4- G. Cheddar and G. Tollin, "Environmental Effects on Electron Transfer from Chlorophyll Triplet to Quinone: Role of Dielectric Constant, Viscosity and Quinone Structure in Cellulose Acetate Films," Photobiochem. Photobiophys. 2, 255 (1981).
- 5- J. K. Hurley, F. Castelli and G. Tollin, "Chlorophyll-Quinone Photochemistry in Liposomes: Mechanisms of Radical Formation and Decay," Photochem. Photobiol. 34, 623 (1981).
- 6- J. K. Hurley and G. Tollin, "Photochemical Energy Conversion in Chlorophyll-Containing Lipid Bilayer Vesicles", Solar Energy 28, 187 (1982).
- 7- W. E. Ford and G. Tollin, "Direct Observation of Electron Transfer Across a Lipid Bilayer: Laser Photolysis of an Asymmetric Vesicle System Containing Chlorophyll, Methyl Viologen and EDTA," Photochem. Photobiol. 35, 419 (1982).
- 8- W. E. Ford and G. Tollin, "Chlorophyll Photosensitized Electron Transfer in Phospholipid Vesicle Bilayers: Inside vs. Outside Asymmetry", Photochem. Photobiol. 36, 647 (1982).
- 9- Y. Fang and G. Tollin, "Light-Induced Electron Transfer Reactions Between Chlorophyll and Quinone in Liposomes: Radical Formation and Decay in Negatively and Positively Charged Vesicles", Photochem. Photobiol. 38, 429 (1983).
- 10- W. E. Ford and G. Tollin, "Chlorophyll Photosensitized Vectorial Electron Transport Across Phospholipid Vesicle Bilayers: Kinetics and Mechanism", Photochem. Photobiol. 38, 441 (1983).
- 11- Y. Fang and G. Tollin, "Light-Induced Electron Transfer Reactions Between Chlorophyll and Quinone in Electrically-Charged Vesicles: Effects of Counterions Located in Either the Inner or Outer Aqueous Phases on Radical Formation and Decay", Photochem. Photobiol. 39, 685 (1984).

12- W. E. Ford and G. Tollin, "Chlorophyll Photosensitized Electron Transfer in Phospholipid Bilayer Vesicle Systems: Effects of Cholesterol on Radical Yields and Kinetic Parameters", Photochem. Photobiol. 40, 249 (1984).

13- V. Senthilathipan and G. Tollin, "Light-Induced Electron Transfer Reactions Between Chlorophyll and Electrically Charged Acceptors in Positively and Negatively Charged Lipid Bilayer Vesicles", Photochem. Photobiol. 42, 437 (1985).

14- W. E. Ford and G. Tollin, "Chlorophyll Photosensitized Electron Transfer in Phospholipid Bilayer Vesicle Systems: Correlations Between Kinetic Parameters and Solubilities of Viologen Acceptors", Photochem. Photobiol. 43, 319 (1986).

15- W. E. Ford and G. Tollin, "Chlorophyll Photosensitized Electron Transfer in Phospholipid Bilayer Vesicle Systems: Secondary Oxidation of Reduced Viologen Acceptors by $\text{Ru}(\text{NH}_3)_6^{3+}$ ", Photochem. Photobiol. 43, 467 (1986).

16- V. Senthilathipan and G. Tollin, "Horse Heart Cytochrome c as an Electron Acceptor from Chlorophyll Triplet in Negatively Charged Lipid Bilayer Vesicles", Photochem. Photobiol. 43, 545 (1986).

17- V. Senthilathipan and G. Tollin, "Spinach Plastocyanin as an Electron Acceptor from Membrane-bound Chlorophyll Triplet State in Positively Charged Lipid Bilayer Vesicles", Photochem. Photobiol., 46, 537 (1987).

18- S. Rong and G. Tollin "Chlorophyll Photosensitized Electron Transfer in Phospholipid Vesicle Systems: Comparison of Inside-Outside Asymmetry Between Large and Small Unilamellar Vesicles", Photochem. Photobiol., 47, 277 (1988).

19- J. Szebeni and G. Tollin, "Interaction of Cytochrome c With Liposomes: Covalent Labelling of Externally-Bound Protein by the Fluorescent Probe, Azidonaphthalenedisulfonic Acid, Enclosed in the Inner Aqueous Compartment of Unilamellar Vesicles", Biochim. Biophys. Acta, 932, 153 (1988).

20- J. Szebeni and G. Tollin, "Some Relationships between UV Light and Heme Protein - Induced Lipid breakdown in Liposomes, as reflected by fluorescence changes. The Effect of Negative Surface Charge", Photochem. Photobiol., 47, 475 (1988).

21- Y. Fang and G. Tollin, "Electron Transfer Reactions occurring upon Laser Flash Photolysis of Phospholipid Bilayer Systems Containing Chlorophyll, Benzoquinone and Cytochrome c. I. Negatively-charged vesicles", Photochem. Photobiol., 47, 741 (1988).

22- Y. Fang and G. Tollin, "Electron Transfer Reactions Occurring upon Laser Flash Photolysis of Phospholipid Bilayer Systems Containing Chlorophyll, Benzoquinone and Cytochrome c. II. Electrically Neutral and Positively-Charged Vesicles", Photochem. Photobiol., 47, 751 (1988).

23- V. Senthilathipan and G. Tollin, "Laser Flash Photolysis as a Probe of Redox Protein-Membrane interactions: Effect of Binding of Spinach Plastocyanin and Horse Cytochrome c to Lipid Bilayer Vesicles on the Kinetics of Reduction by Flavin Semiquinone". Biochemistry, 28 1133 (1989).

24- S. Rong, R. K. Brown and G. Tollin, "Kinetics of Chlorophyll Photosensitized Electron Transfer Reactions in Phospholipid Bilayers: Electrostatic Effects of Surface Charge and Valinomycin Addition", Photochem. Photobiol., 49, 107 (1989).

25- V. Senthilathipan and G. Tollin, "Spinach Ferredoxin Reduction by Membrane-Bound Chlorophyll via a Diquat Mediator", Photochem. Photobiol., 49, 345 (1989)

26- V.G. Chamupathi and G. Tollin, "Quinones as Mediators of Electron Transfer from Membrane-Bound Chlorophyll to Cytochrome c in the Aqueous Phase in Lipid Bilayer Vesicles: Synergistic Enhancement of Cytochrome c Reduction by Lipophilic/Hydrophilic Quinone Pairs", Photochem. Photobiol., 51, 611 (1990).

27- V.G. Chamupathi, D.M. Moezzi and G. Tollin, "Redox Proteins from Chlorophyll Triplet State in Lipid Bilayer Vesicles: Kinetics of Reduction of Membrane Reconstituted Cytochrome c Oxidase Mediated by 2,5-Di-*t*-Butyl Benzoquinone and Cytochrome c", Photochem. Photobiol., 52, 883 (1990).

28- G. Tollin, "Chlorophyll Photochemistry in Microheterogeneous Media" in The Chlorophylls, H. Scheer, ed., CRC Press, Boca Raton, p. 317, 1991.

29- Z.G. Zhao & G. Tollin, "Chlorophyll Photosensitized Electron Transfer Reactions in Lipid Bilayer Vesicles: Vectorial Electron Transfer Across the Bilayer from Reduced Cytochrome c in the Inner Compartment to Oxidized Ferredoxin in the Outer Compartment", Photochem. Photobiol., 54, 113 (1991).

30- Z.-G. Zhao & G. Tollin, "Chlorophyll Photosensitized Electron Transfer Reactions in Lipid Vesicles: Enhancement in Yield of Vectorial Electron Transfer from Reduced Cytochrome c to Oxidized Ferredoxin by Addition of Valinomycin Plus Potassium Ion", Photochem. Photobiol., 54, 827 (1991).

31- Z. Salamon & G. Tollin, "Reduction of Cytochrome c at a Lipid Bilayer Modified Electrode", Bioelectrochem. Bioenerg., 25, 447 (1991).

- 32- Z. Salamon & G. Tollin, "Interfacial Electrochemistry of Cytochrome c at a Lipid Bilayer Modified Electrode: Effect of Incorporation of Negative Charges into the Bilayer on Cyclic Voltammetric Parameters", Bioelectrochem. Bioenerg., 26, 321 (1991).
- 33- Z.-G. Zhao & G. Tollin, "Chlorophyll Photosensitized Electron Transfer Reactions in Lipid Bilayer Vesicles: Generation of Proton Gradients Across the Bilayer Coupled to Quinone Reduction and Hydroquinone Oxidation", Photochem. Photobiol., 55, 611 (1992).
- 34- Z. Salamon & G. Tollin, "Cyclic Voltammetric Behavior of (2Fe-2S) Ferredoxins at a Lipid Bilayer Modified Electrode", Bioelectrochem. Bioenerg., 27, 381 (1992).
- 35- Z. Salamon & G. Tollin, "Direct Electrochemistry of Spinach Plastocyanin at a Lipid Bilayer Modified Electrode: Cyclic Voltammetry as a Probe of Membrane-Protein Interactions", Arch. Biochem. Biophys., 294, 382 (1992).
- 36- Z.G. Zhao & G. Tollin, "Vectorial Electron Transport Across Lipid Vesicle Membranes Driven by Two Photoreactions. I. EDTA as an Electron Donor via Flavin Triplet State in the Inner Compartment and Cytochrome c as an Electron Acceptor from Chlorophyll Triplet State in the Outer Compartment", Photochem. Photobiol., 57, 331 (1993).
- 37- Z.G. Zhao & G. Tollin, "Vectorial Electron Transport Across Lipid Vesicle Membranes Driven by two Photoreactions. II. Photochemical Regeneration of Reduced Cytochrome c by Flavin Triplet State and EDTA in the Inner Compartment and Reduction of Ferredoxin by Chlorophyll Triplet State in the Outer Compartment", Photochem. Photobiol., 57, 338 (1993).
- 38- Z. Salamon, J. T. Hazzard & G. Tollin, "Direct Measurement of Cyclic Current-Voltage Responses of Integral Membrane Proteins at a Self-Assembled Lipid Bilayer Modified Electrode: Cytochrome f and Cytochrome c Oxidase", Proc. Natl. Acad. Sci. U.S.A. 90, 6420 (1993).
- 39- G. Tollin, Z.-G. Zhao & Z. Salamon, "Biomimetic Systems for Photochemical Energy Conversion Based Upon Green Plant Photosynthesis", Proc. Symp. on Plant Growth & Environment, Suwon, Korea, 1-17 (1993).
- 40- Z. Salamon & G. Tollin, "Chlorophyll-Photosensitized Electron Transfer Between Cytochrome c and a Lipid-Modified Transparent Indium Oxide Electrode", Photochem. Photobiol. 58, 730 (1993).

*Reprints removed
and cited separately*