Progress Report

MAGNETIC RESONANCE AND OPTICAL SPECTROSCOPIC
STUDIES OF CAROTENOIDS

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No unexpended funds are anticipated to be left at the end of the current budget period. No change has occurred from the aims proposed in the original application.

PLANNED FOR NEXT YEAR

Work will continue as outlined in our original proposal with emphasis on using simultaneous electrochemistry EPR, and optical methods, variable temperature electrochemistry. using microelectrodes and fast scans to examine the more unstable intermediates formed upon electrochemical oxidation of synthetically prepared carotenoids. We expect to complete our current joint project with R. Fessenden, and T. Cotton this coming year. No real problems have occurred this year and none are anticipated during the next year.

INTRODUCTION

The fundamental goals of this project are (1) to understand the role of a host matrix in the formation and decay mechanisms of carotenoid cation radical and dication and (2) to determine the special properties of carotenoids that enable them to serve as photoprotective agents in photosynthesis and as possible components in electron transfer processes.

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RESULTS OF WORK TO DATE

Papers published, accepted or submitted on the work supported by the grant this year (#1-5), titles of papers in preparation (#6-13) and abstracts (#14-23) to papers at meetings are listed starting on page 5. A brief summary is given below of the new results in each area. The expertise of Dr. A. Jeevarajan in EPR and laser spectroscopy, free radical chemistry and computer networking; the synthetic ability of Dr. Elli Hand; the efforts of three hard working graduate students; C. C. Wei, J. Jeevarajan and G. Gao and the assistance of two undergraduate summer students Mark Feldman and Sherri McFarland have resulted in a large number of significant results. It is now clearer how carotenoids function as photoprotective and antioxidant materials. A number of papers are now in preparation (#6-13) and two Ph.D. dissertations will be completed during the next year.

Joint projects with other labs have been very productive. The study of short lived carotenoid cation radicals (CAR^+) in CCl_4 solution was carried out using the transient 35 GHz EPR-pulsed laser facility located in Professor Malcolm Forbes lab at the University of North Carolina. It was determined that the $\text{CCl}_4^- \text{CAR}^+$ species was formed photochemically and that the electron transfer to the solvent occurs from the excited single state of β -carotene. A paper has been submitted to Chem. Phys. Letters (#5) and presentations 22 and 23 will be given. An abstract for paper #5 is attached.

Optical double pulse experiments were carried out in Professor Richard Fessenden's Lab at The University of Notre Dame attempting to unequivocally establish the optical absorption spectra of the carotenoid dication using a two pulse laser experiment. Light from the second pulse at 1064 nm (Nd-YAG) is indeed absorbed. New detectors operating in the 900-1100 nm region are now being installed so spectra can be recorded in this region (preliminary results were reported at the DOE Research Conference #21 and preparation #11).

Resonance Raman measurements carried out at Professor Cotton's lab (Ames, IA) have shown that *in situ* electrochemically formed carotenoid cation radical

exhibits changes in the C-C and C=C stretch frequency indicating delocalization of the unpaired electron through the entire carbon back-bone (paper #10). These results are consistent with our previous EPR and ENDOR results (see papers #1, 2, 3 and presentations #14, 18, 20, 23). Pulsed ENDOR measurements were carried out at the Bruker headquarters in Karlsruhe, Germany on the cation radical of canthaxanthin and apocarotenal on silica alumina solid support in an attempt to gain better ENDOR resolution. Strong evidence exists that ^{13}C couplings were observed. A complete analysis is planned for this next year.

Photoconductivity measurements were carried out for apocarotenal, canthaxanthin and the dicyano substituted single crystals in Dr. Angerhofer's lab at the Universitat Stuttgart in Germany. It was unfortunate that the canthaxanthin crystals grown in our lab were not of such size to give good enough spectra to provide information about the origin of the photoconduction spectrum in the 600 - 850 nm - reported earlier for β -carotene. However large flat crystals of apocarotenal and the dicyano substituted carotenoid gave interesting results. Delays have occurred in this project because Dr. Angerhofer has joined the University of Florida faculty as an assistant professor. He plans to set-up the same system at Florida so further measurements can be done in the future.

Simultaneous electrochemical and Electron Paramagnetic Resonance Studies have been carried out for cation radicals and dications of carotenoids with donor/acceptor substituents (papers #1, 2, 12, presentations #18, 19, 20), acetylenic carotenoids (#4), and keto and hydroxyl carotenoids (#1). Reprints have been attached for #1, 2 and the abstracts for #4 and #5 are given below. A reprint (#3) is attached detailing our ENDOR study of UV photolysis of carotenoids absorbed on silica gel. The EPR parameters ΔH_{pp} and g are found to be the same irrespective of the donor or acceptor character of the terminal substituents.

Simultaneous variable temperature electrochemical and optical measurements have shown that cis isomers are formed during the electrochemical oxidation (papers 7, 13). A number of the isomers have been isolated by HPLC techniques and

identified by NMR and optical measurements (papers 8, 13 and presentations 17). AM1 molecular orbital calculations have been carried out. From the relative energetics of the neutral carotenoid and its cation radicals and dications plausible isomerization mechanisms have been deduced (paper #8 and presentation #21) and it is now clear why isomers of carotenoids are found in photosynthetic materials.

The cation radical and dication of 7,7'-diapo-7,7'-diphenylcarotenoid formed electrochemically in CH_2Cl_2 solution was observed to adsorb very strongly on typical electrodes (Pt, carbon paste, glassy carbon etc). This unusual behavior has enabled us to examine the adsorption character of these carotenoid cation radical and dications in CH_2Cl_2 on metal surfaces (paper #6, and presentation 15). Evidence for a weak tendency for all carotenoid cation radicals and dications to adsorb on platinum metal has been detected by exhaustive simulation of CV spectra for a large number of carotenoids (papers #4, 12 and presentation 16) using the Digism[®] electrochemical program developed by Steve Feldberg (Brookhaven) and marketed by Bioanalytical Systems.

Possibly the most important recent result has been the determination of the optical absorption spectra for the dication of canthaxanthin and β -carotene (paper #9 and presentation 20). The adsorption maximum and the extinction coefficient have been determined both by variable temperature electrochemical and chemical (oxidation by ferric chloride) methods. Quantitative agreement by both methods occurs for canthaxanthin. These very important measurements should now settle the controversy in the literature between conducting polymer and radiation/photochemists about the optical spectra and extinction coefficient for the dications of carotenoids.

DISCLAIMER

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PAPERS PUBLISHED, ACCEPTED OR SUBMITTED FOR PUBLICATION THIS PAST YEAR (1994- 1995)

- ✓1. A. S. Jeevarajan, M. Khaled and L. D. Kispert, "Simultaneous Electrochemical and Electron Paramagnetic Resonance Studies of Keto and Hydroxyl Carotenoids." *Chem. Phys. Lett.*, 219, 427 (1994).
- ✓2. A. S. Jeevarajan, M. Khaled and L. D. Kispert, "Simultaneous Electrochemical and Electron Paramagnetic Resonance Studies of Carotenoids: Effect of Electron Donating and Accepting Substituents," *J. Phys. Chem.* 98, 7777 (1994).
- ✓3. L. Piekara-Sady, A. S. Jeevarajan, L. D. Kispert, E. G. Bradford, and M. Plato, ENDOR Study of the (7',7'-Dicyano)- and (7'-Phenyl)-7'-apo- β -carotene Radical Cations formed by UV Photolysis of Carotenoids adsorbed on Silica Gel," *J. Chem. Soc. Faraday Trans.* 41, 0000 (1995).
4. J. A. Jeevarajan, A. S. Jeevarajan and L. D. Kispert, "SE-EPR, CV and AM1 Studies of Acetylenic Carotenoids, *J. Phys. Chem.* submitted - in revision.
5. A. S. Jeevarajan, L. D. Kispert, N. I. Avdievich and M. D. E. Forbes, "Role of Excited Singlet State in the Photooxidation of Carotenoids: A Time Resolved Q-band EPR Study," *Chem. Phys. Letters*, submitted

IN PREPARATION

6. G. Gao, A. S. Jeevarajan and L. D. Kispert, "Cyclic Voltammetry and Spectroscopic Studies of Dication and Cation Radical Adsorption Behavior for 7,7'-Diapo-7,7'-Diphenyl β -carotene,"
7. C. C. Wei, G. Gao and L. D. Kispert, "Cis/trans Isomerization of Canthaxanthin by Electrolysis,"
8. G. Gao, C. C. Wei, A. S. Jeevarajan and L. D. Kispert, "Electrochemical Trans-cis Isomerization of Carotenoids and the Study of the Isomerization Mechanism by HPLC, *in situ* Spectroelectrochemical Techniques and AM1 Calculations,"
9. J. A. Jeevarajan, C. C. Wei, A. S. Jeevarajan and L. D. Kispert, "Optical Absorption Spectra of Carotenoid Dications Formed Chemically and Electrochemically,"
10. A. S. Jeevarajan, L. D. Kispert, G. Chumanov, C. Zhou and T. M. Cotton, "Resonance Raman Study of 8'-apo- β -carotene-8'-al Cation Radical,"
11. A. S. Jeevarajan, L. D. Kispert and R. W. Fessenden, "Optical Absorption Spectrum of the Carotenoid Dication Using Optical Double Pulse Techniques,"
12. Ph.D. Dissertation: Judith Jeevarajan, "Electrochemical and Optical Study of Carotenoid Cation Radicals, Dications and Trication Radicals," Dec., 95.

13. Ph.D. Dissertation: C. C. Wei, "Isolation, and Identification of Carotenoid Cis Isomers Formed Electrochemically,"

Presentations at Conferences

14. "An ENDOR Study of Synthetic Carotenoid Cation Radicals," A. S. Jeevarajan, L. Piekara-Sady, M. Plato and L. D. Kispert at the 36th Rocky Mountain Conference, EPR Symposium July 31 - Aug. 4, 1994.
15. "Adsorption of Carotenoid Cation Radicals on Various Electrodes as Detected by Cyclic Voltammetry," G. Gao and L. D. Kispert at the 46th SERMACS Meeting, Birmingham, AL, Oct. 16-19, 1994.
16. "Use of Digisim® in the Analysis of Complex CVS of Oxidized Carotenoids," J. A. Jeevarajan, and L. D. Kispert at the 46th SERMACS Meeting, Birmingham, AL, Oct. 16-19, 1994.
17. "Thermal Isomerization of a Carotenoid with Strong Acceptor Groups: HPLC Separation Techniques," C. C. Wei and L. D. Kispert at the 46th SERMACS Meeting, Birmingham, AL, Oct. 16-19, 1994.
18. "SEEPR, CV and ENDOR Analyses in the Oxidation of Carotenoids," A. S. Jeevarajan, L. D. Kispert at the 46th SERMACS Meeting, Birmingham, AL, Oct. 16-19, 1994.
19. "Electrochemistry of Carotenoids," M. Feldman and L. D. Kispert at the 46th SERMACS Meeting, Birmingham, AL, Oct. 16-19, 1994.
20. "EPR, ENDOR, SEEPR and Spectroelectrochemical Studies of Carotenoid Cation Radicals," A. S. Jeevarajan and L. D. Kispert at the 26th Southeastern Magnetic Resonance Conference, Chapel Hill, NC, Oct. 23-25, 1994.
21. "Properties of Carotenoid Cation Radicals and Dications as Deduced by Optical, Electrochemical and Magnetic Resonance Measurements," A. S. Jeevarajan, G. Gao, C. C. Wei, J. A. Jeevarajan, E. Hand and L. D. Kispert at the 19th DOE Solar Photochemistry Research Conference, June 4-8, 1995 Tamiment, PA.
22. "A Time-Resolved EPR (35 GHz) Study of Carotenoid Cation Radicals," A. S. Jeevarajan, N. I. Avdievich, M. D. E. Forbes and L. D. Kispert at the 37th Rocky Mountain Conference, EPR Symposium, July 23-28, 1995, Denver, CO.
23. "A Time-Resolved EPR (35 GHz) Study of Carotenoid Cation Radicals," A. S. Jeevarajan, N. I. Avdievich, M. D. E. Forbes and L. D. Kispert at the 37th Rocky Mountain Conference, EPR Symposium, July 23-28, 1995, Denver, CO.