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Technical Progress Report

Investigation of the Triplet State of Chlorophylls

Progress report for the period
May 1, 1980 through April 30, 1981

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I. Abstract

The triplet state of chlorophyll has been utilized as a nondestructive probe into the structural and dynamical nature of the photosynthetic apparatus. During the past year, using zero-field triplet state optically detected magnetic resonance spectroscopy, our research program has examined chlorophyll aggregates in both their natural environment in vivo and in model complexes in vitro. Our research effort has focused on subchloroplast particles extracted from green plants, on chlorophyll monolayers, and on isolated chlorophyll molecules bound to both simple ligands and to complete proteins. The overall aim of the research program has been to detail the physical features and interactions of the photosynthetic pigment systems through examination of structural subunits of the photosynthetic apparatus and model systems which represent them.

II. Progress Report (5/1/80 - present)

Our research program over the past support period (5/1/80 - 4/30/81) has continued a parallel effort to investigate chlorophyll aggregates in vitro and in vivo by zero-field triplet state ODMR spectroscopy. As has been the basic approach of our research program over the past several years, the structural features, stabilizing interactions and excited state dynamics of these systems have been investigated through use of a photoexcited triplet state generated as a nondestructive probe within the systems and the triplet state properties utilized to interpret their physical makeup. The value of ODMR spectroscopy, combining high resolution and high sensitivity is particularly apparent when one considers the molecular complexity of the systems discussed below.

Specifically, the experiments to date have resulted in the following developments:

A. In Vivo Chlorophyll.

In vivo chlorophyll aggregates exist in several localized sites within the chlorophyll units; those which have been best characterized by bioanalytical methods are the chlorophyll-protein complexes containing photosystem 1 (PS I), the complex containing photosystem 2 (PS II), and the antenna light-harvesting chlorophyll protein (LHCP) complex (1). Several procedures have emerged recently to prepare subchloroplast particles enriched in each of these three components, and we have continued a major effort in our group during the past year to investigate several of these preparations by triplet state ODMR spectroscopy, an undertaking that was not without its problems. The most practical finding that emerged from the ODMR work (which is a technique very sensitive to small changes in the system being examined) was that the preparative methods employed had a measurable

effect on the spectra observed. Unlike our ODMR work on bacterial reaction centers which showed a uniform spectrum from system to system (2), different subchloroplast preparations produced a non-constant system for investigation. For that reason it is not yet clear how much structural information can be extracted from the ODMR spectra; we still require development of better separation and purification techniques, an area of intense activity among several photosynthesis groups, as reported at the most recent International Photosynthesis Congress (3).

Our main effort focused on two alternative preparation, the procedures of Bengis and Nelson (4) and those of Satoh and Butler (5). Both approaches result in fractions enriched in PS I, PS II, and LHCP.

The Bengis and Nelson preparations resulted in the strongest ODMR spectra, observable from each of the three sets of enriched particles; those preparations also provided the most significant spectroscopic problems. From the fluorescence spectra alone one could observe that the preparations were not stable; the positions and relative intensities of the fluorescence peaks varied depending on the stage of particle preparation and with the length of storage time (even at low temperatures). The fluorescence changes were also accompanied by changes in the ODMR frequencies observed on the main fluorescence bands. Although the ODMR spectra observed on the lowest energy ($\lambda = 735$ nm) fluorescence band correlated well with the frequencies and dynamics observed in vitro for the simple ligand-bound chlorophyll dimer (6), the variable character of the preparation prevents a clear-cut conclusion about the PS I reaction center structure.

The Satoh and Butler preparations, in contrast, produced more stable spectroscopic systems, but weaker ODMR spectra. The existence of well-defined and characterizable fluorescence spectra for each of the fractions separated in this preparation suggests that these systems represent the better chemical choice for investigation. In addition, tests for PS I reaction center activity in the Satoh and Butler preparation (done by both absorbance and conventional EPR spectroscopy)

indicate a better quality procedure. The ODMR spectra observed for the LHCP complex from the Satoh and Butler preparation reproduce some of the earlier results from the Bengis and Nelson systems, namely the appearance (at a detection wavelength of 670 nm) of chlorophyll a triplet state microwave transitions, but with slower intersystem crossing rate constants than has been observed for the chlorophyll a molecule complexed with water in a hydrocarbon solvent (7). We are presently investigating this complex further to determine whether the slower dynamics are due to energy transfer through the chlorophyll b pigments (as was our original hypothesis) or are due to the specific effect of protein binding affecting the rate of intersystem crossing of the chlorophyll a, changing its value from what is observed in vitro.

B. In Vitro Chlorophyll

Our work on chlorophyll aggregates in vitro has focused on the triplet state properties of the following systems:

1. Spin delocalization in the pyrochlorophyll a covalently linked dimer.
2. Monolayers and multilayers of chlorophyll a and chlorophyll b.
3. Ligand effects on the chlorophyll a intersystem crossing rates.

One of the most important experiments performed in our laboratory during the past year was the direct measurement of the spin delocalization in the triplet state of a water-linked chlorophyll dimer. The importance of the experiment lies in the fact that it established the appropriateness of the triplet exciton approach to determining molecular orientations in the chlorophyll dimer, in the test case where the dimer molecules are in a parallel configuration. By using high-field EPR to measure the proton hyperfine interactions in the folded (dimeric) and unfolded (monomeric) pyrochlorophyllide a covalently linked dimer, we determined that the triplet unpaired electrons were delocalized (within experimental error) equivalently distributed over the two molecular units in the

water-linked dimeric configuration. These hyperfine measurements support an exciton description of the chlorophyll dimer triplet state, properly treated in the strong spin coupling limit. Properties of the triplet state may, therefore, be utilized within the strong spin coupling limit to calculate geometrical features of the chlorophyll dimer both in model systems in vitro and for chlorophyll aggregates in vivo.

Work on mixed monolayers of chlorophyll a and chlorophyll b, systems being prepared for our experiments by collaborative agreement with the University of Quebec, has been initiated. Early results indicate that the precise pigment mixture has a profound effect on the triplet concentrations present in the sample. Our initial experiments were run on systems containing 95% or higher concentrations of chlorophyll b and 5% or less chlorophyll a, the idea being to create a mixed crystal situation in which the excitation energy would ultimately trap out in the lowest energy chlorophyll a triplet state. Even at the low temperatures of our experiments (2 K), no chlorophyll a triplets were observed, suggesting that supertrap migration among the chlorophyll b pigment molecules may be more efficient than local trapping. Mixtures with approximately equal components of chlorophyll a and chlorophyll b seem to avoid this difficulty and are presently under study for ODMR spectra and energy transfer dynamics.

We have also returned to the triplet state of the chlorophyll monomer, prompted by the in vivo results described above and by our collaborative work with Prof. Steve Boxer (Stanford Univ.) on the reconstituted myoglobins. There is now significant evidence that both the number of ligands bound and the exact positioning of the ligand effects a significant change in the intersystem crossing rates of chlorophyll, while showing little effect on the zero-field splittings. This result had been suggested in some early ODMR results on chlorophyll by Schaafsma and coworkers (8), but became more evident in the chlorophyll-reconstituted myoglobins. We have, therefore, initiated a program to examine the effect

of specific ligands on the intersystem crossing dynamics in chlorophyll. The first results show that monohydrated and bihydrated chlorophyll differ by a factor of two to ten in their rates of intersystem crossing from the individual triplet spin sublevels. These ligand effects are centrally important to be understood, since the comparison between chlorophyll triplet properties in vitro in model systems and those in vivo depends critically on this information for proper interpretation.

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III. Personnel Involved in Research Program (5/1/80 - present)

1. Dr. Willem R. Leenstra - postdoctoral research associate working 50% of full time on this research. Left our lab on 8/31/80 to become Assistant Professor of Chemistry, University of Vermont, Burlington, Vt.
2. Pradip Mitra - graduate research assistant
Graduate student, working 100% of time on this research.
Ph.D. candidate, degree expected in 1981.
3. Surya Jaganathan - graduate research assistant
Graduate student, working 100% of time on this research.
Ph.D. candidate, degree expected in 1981.
4. K. Vinodgopal - graduate research assistant
Graduate student, working 50% of time on research, 50% of time on course work.
Ph.D. candidate, degree expected in 1982.
5. Eugene Hanlon - graduate research assistant
Graduate student, working 50% of time on research, 50% of time on coursework.

IV. Publications Acknowledging DOE Support

Publications 5/1/75 - present

- R.H. Clarke, R.E. Connors, J.R. Norris and M.C. Thurnauer
"Optically Detected Zero-Field Magnetic Resonance Studies of the Photoexcited Triplet State of the Photosynthetic Bacterium Rhodospirillum Rubrum"
J. Am. Chem. Soc., 97, 7178 (1975).
- R.H. Clarke and H.A. Frank
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J. Chem. Phys., 65, 39 (1976).
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"The Triplet State of Chlorophylls"
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- R.H. Clarke and H.A. Frank
"Investigation of the Effect of Metal Substitution on the Triplet States of Chlorophyll by Optically Detected Zero-Field Magnetic Resonance"
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"Structural Aspects of the Reaction Center of Photosynthetic Bacteria Calculated from Triplet State Zero-Field Splittings"
F.E.B.S. Letters, 82, 155 (1977).
- R. H. Clarke, R. E. Connors and H. A. Frank
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"Observation of a Triplet State in Chlorophyll Protein 668 via Optically Detected Magnetic Resonance"
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"The Triplet State of the Chlorophyll Dimer"
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"A Comparative Study of Dimerization of Chlorophylls and Pheophytins by Fluorescence and ODMR"
Chem. Phys. Letters, 68, 65 (1979).
- R. H. Clarke, S. P. Jagannathan and W. R. Leenstra
"Optical-Microwave Double Resonance Spectroscopy of In Vivo Chlorophyll"
Lasers in Photomedicine and Photobiology, R. Pratesi and C. Sacchi, eds. (Springer-Verlag, Berlin, 1980) P.171.
- R. H. Clarke, S. P. Jagannathan and W. R. Leenstra
"Optical-Microwave Double Resonance Spectroscopy of In Vivo Chlorophyll"
Photochem. Photobiol., 32, 805 (1980).

Publications accepted for publication and in press

- R. F. Clark and R. H. Clarke
"Electron Delocalization in the Lowest Triplet State of the Covalently Linked Pyrochlorophyllide A Dimer"
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"Optically Detected Zero-Field Triplet State Spectroscopy of In Vivo Chlorophyll"
Proceedings of the Fifth International Congress on Photosynthesis, G. A. Akoyunoglou, ed., to be published.
- R. H. Clarke
"The Chlorophyll Triplet State and the Structure of Chlorophyll Aggregates"
Light Reactions in Photosynthesis, F. Fong, ed., (Springer-Verlag, Berlin, 1981) in press.
- R. H. Clarke, ed.
Triplet State ODMR Spectroscopy: With Applications to Biophysical Systems
(Wiley-Interscience, New York, 1981) in press.

Talks presented on DOE research program at major international meetings
(5/1/80 - present)

Fourth Gordon Research Conference on Magnetic Resonance in Medicine and
Biology, Tilton, N. H., August 10-15, 1980.

"Optically Detected Zero-Field Magnetic Resonance Spectroscopy
Applied to Biomolecular Systems"

Fifth International Congress on Photosynthesis, Kallithea, Greece,
September 7-13, 1980.

"Optically Detected Zero-Field Triplet State Spectroscopy of In Vivo
Chlorophyll"

V. Budget (5/1/80 - 4/30/81)

It is projected that all funds provided for this contract period will be fully expended in the course of this research program, and no funds will remain after April 30, 1981.