

# MASTER

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Technical Progress Report

Investigation of the Triplet State of Chlorophylls

Progress Report for the period  
May 1, 1979 through April 30, 1980

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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, both in vitro and in isolated in vivo subunits. During the past year, using zero-field triplet state optically detected magnetic resonance spectroscopy, our research program has examined triplet energy transfer between photosynthetic pigments in solution; has isolated and studies subchloroplast particles enriched with photosystem I and the light harvesting chlorophyll protein (antenna) system; and has examined model chlorophyll-protein systems, specifically chlorophyll protein 668 and the reconstituted myoglobins. 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 (5/1/79 - present)

Our research program over the past support period (5/1/79 - 4/30/80) has been focussed on three areas, all involving zero-field ODMR spectroscopy in the triplet state of the chlorophyll pigment. The areas of concentration have been:

1. Chlorophyll a - chlorophyll b triplet energy transfer in frozen solution.
2. Makeup of photosystem I (PSI) and light harvesting chlorophyll protein (LHCP) particles.
3. Triplet state spectra of model chlorophyll-protein systems.

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 listed above.

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

1. Chlorophyll a - chlorophyll b triplet energy transfer

Chronologically, the motivation to investigate triplet transfer between the two commonly occurring photosynthetic pigments originated from the discovery that such processes appeared to occur in vivo with considerable efficiency (vide infra - LHCP protein). In order to examine the specific nature of the triplet interaction between the triplet donor (chl b) and triplet acceptor (chl a) we examined the system containing both pigments in frozen solutions of highly dried and purified toluene, made up to concentrations of  $\sim 10^{-5}$  M in both pigments. Using light excitation tuned selectively to either the chlorophyll a or b Soret absorption band, the

ODMR spectra were recorded on all observable fluorescence bands. The discovery that photoexcitation into chlorophyll b (less than 1% chlorophyll a absorption) produced a strong ODMR spectrum of chlorophyll a indicated significant triplet transfer from the chlorophyll b pigment to that of chlorophyll a. This result is experimentally quite dramatic, since the chlorophyll a ODMR intensity is enhanced by about an order or magnitude over that observed for chlorophyll a when directly excited, or in the absence of the chlorophyll b pigment. Although several experiments still remain to be tried on this system (particularly, measurements of the dynamics of buildup of the ODMR signal for chlorophyll a when exciting through chlorophyll b) the result suggests that triplet energy transfer is a nonnegligible process in regions of the photosynthetic apparatus (e.g., the antenna system) where both pigments are present. Further work is in progress to determine distances between pigments and relative orientations involved in the transfer process.

## 2. PSI and LHCP particles

Having established the basic structural features in vitro for the water linked chlorophyll dimer in our previous work [J. Am. Chem. Soc., 101, 2416 (1979)] it was of direct interest to attempt to find evidence via triplet state ODMR spectroscopy, for the presence of such dimer structures (or, more properly, such ODMR frequencies) in the chlorophyll-containing constituent units of naturally occurring photosynthetic systems. Using procedures of Bengis and Nelson and those of Butler and coworkers we isolated subchloroplast particles enriched in PSI, PSII and LHCP. Our efforts to date have focused on the PSI and LHCP particles, since they are the most reliably obtained units in such preparations, although efforts continue to isolate and purify PSII particles. ODMR transition frequencies and spin sublevel decay rates were measured for each fluorescence peak (and, systematically, throughout each fluorescence band) observable for the PSI and LHCP particles. For the

PSI particles ODMR transitions could be detected on the 730 nm fluorescence band, and for the LHCP particles ODMR transitions were found when optically detecting at 680 nm. Both sets of transitions were at frequencies in the region of chlorophyll a in simple organic solvents; those detected in the PSI system were 50-100 MHz lower in frequency than those observed for the LHCP.

The most interesting feature of the results obtained was in the dynamics observed. For the LHCP, the only system for which complete dynamical information is presently available, our experiments found that, while the frequencies observed were close to those for a chlorophyll a species, the dynamics observed were clearly not. We have measured the spin sublevel decay rates for the triplet states associated with the 680 nm fluorescence band to determine which pigments were involved. The rates observed at 680 nm are consistently smaller than is observed for chlorophyll a in hydrocarbon solvents by several laboratories ( $k_x = 661$ ,  $k_y = 1255$ ,  $k_z = 241$ ). In fact, the values are remarkably similar to those observed for the ODMR spectra detected at 650 nm, assigned as chlorophyll b. Since the chlorophyll b rates show that, under the sample conditions, the usual molecular rates can be obtained, one must conclude that the makeup of the solubilized species fluorescing at 670 nm is not simple free chlorophyll a molecule. The similarity in dynamical features observed at 650 and 670 nm leads us to consider the possibility that the 680 nm fluorescence originates in fractionated particles of the LHCP complex. Such particles would contain both chlorophyll a and b, interacting with a protein environment. Since the chlorophyll a triplet state is known to be lower in energy than that for chlorophyll b, the triplet excitation within the 670 nm species may be trapped at chlorophyll a sites, providing chlorophyll a ODMR frequencies, but may be populating the chlorophyll a traps via energy transfer from nearby chlorophyll b pigments. Thus, the dynamics observed would reflect the longer-lived chlorophyll b species as it transferred its triplet energy, rather than the purely intramolecular

$T_1 \rightarrow S_0$  relaxation of the shorter-lived chlorophyll a. This explanation, if appropriate, would place important restrictions on the relative positioning of the chlorophyll a and b pigment molecules within the LHCP, since triplet energy transfer is well known to be a very short range phenomenon. Tentatively, we interpret our results as consistent with the pigment arrangement for LHCP proposed by Knox and van Metter [Biochem. Biophys. Acta, 462, 642 (1977)], a model obtained by quite different experimental considerations. Obviously, more spectral data is required, both in vitro and in vivo, before the details of such a model may be finalized, but it is highly interesting that two very different approaches point to a similar pigment arrangement.

Of particular interest will be the dynamics observed for the PSI particles, which are relatively free of chlorophyll b. In this case, we expect to recover both chlorophyll a ODMR frequencies and chlorophyll a dynamics.

### 3. Triplet state spectra of model chlorophyll-protein systems

In order to obtain a detailed picture of the effect of protein interactions on the chlorophyll triplet state, interactions which clearly are important to any interpretation of chlorophyll ODMR spectra in vivo, we have examined two model chlorophyll-protein systems in our research program this year. The two systems are:

- a. chlorophyll protein 668 (CP 668)
- b. myoglobin reconstituted with ethyl chlorophyllide

The CP 668 system, provided to us by Professor William Hagar (U. Mass - Boston), is a useful model system for several reasons: it is water soluble, therefore easily extracted and purified; it has been reported to be photoactive; it contains both chlorophyll a and chlorophyll b pigments in its protein complex. ODMR of this system provided one set of strong transitions, unchanged by photoreaction. Unlike the results obtained for PSI or LHCP particles, both the ODMR frequencies and inter-



system crossing dynamics for CP 668 were close to the values observed for in vitro chlorophyll b. We have concluded that, in CP 668, the triplet excitation is trapped at chlorophyll b sites which are of too large a distance for energy transfer to the lower energy chlorophyll a triplet state. The function of CP 668 in vivo has yet to be established, but these results, in comparison with LHCP, suggest that pigment positioning within the protein environment may be critical in effecting its in vivo mechanisms.

In our most recent work, done in collaboration with Professor Steven Boxer (Stanford), we have examined by ODMR a highly interesting model chlorophyll-protein system - the myoglobin molecule in which the same unit has been removed and replaced with an ethyl chlorophyllide chromophore. These systems, loosely referred to as "chloroglobins", provide the first ordered environment in which to study the details of chlorophyll-protein configurations and the interactions which stabilize them. To date we have observed two ODMR transitions in chloroglobin, one of which is sharp and structureless, and the second which shows three hyperfine components. Interestingly, this same ODMR structure is observed for chlorophyll a when observed in toluene solution. At this point it is premature to draw configurational conclusions from our initial ODMR data, but we expect this system to provide important information on one of the most widely discussed, but least understood, physical features of chlorophylls in photosynthetic units in vivo.

III. Personnel Involved in Research Program (5/1/79 - present)

1. Dr. Willem R. Leenstra - postdoctoral research associate  
working 50% of full time on this research.
2. David R. Hobart - graduate research assistant  
Graduate student, working 100% of time on this research.  
Ph.D. candidate. Degree awarded in January, 1980.
3. Pradip Mitra - graduate research assistant  
Graduate student, working 100% of time on this research.  
Ph.D. candidate, degree expected in 1980-81.
4. Surya Jaganathan - graduate research assistant  
Graduate student, working 100% of time on this research.  
Ph.D. candidate, degree expected in 1980-81.
5. K. Vinodgopal - graduate research assistant  
Graduate student, working 50% of time on research, 50% of  
time as teaching assistant.  
Ph.D. candidate, degree expected in 1981.
6. David Michaels - graduate research assistant  
Graduate student, working 50% of time on research, 50% of  
time as teaching assistant.
7. Robert Clark - undergraduate research assistant  
B.A. degree expected 1980.

IV. Publications, Presentations and Theses 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  
"Triplet State Radiationless Transitions in Polycyclic Hydrocarbons"  
J. Chem. Phys., 65, 39 (1976).
- R. H. Clarke, R. E. Connors, T. J. Schaafsma, J. F. Kleibeuker and R. J. Platenkamp  
"The Triplet State of Chlorophylls"  
J. Am. Chem. Soc., 98, 3674 (1976).
- R. H. Clarke, R. E. Connors and H. A. Frank  
"Investigation of the Structure of the Reaction Center in Photosynthetic Bacteria by Optical Detection of Triplet State Magnetic Resonance"  
Biochem. Biophys. Res. Comm., 71, 671 (1976).
- R. H. Clarke and R. E. Connors  
"Optically Detected Zero-Field Triplet State Magnetic Resonance in Photosynthetic Bacteria"  
Chem. Phys. Letters, 42, 69 (1976).
- R. H. Clarke, R. E. Connors, H. A. Frank and J. C. Hoch  
"Investigation of the Structure of the Reaction Center in Photosynthetic Systems by Optically Detected Zero-Field Magnetic Resonance"  
Chem. Phys. Letters, 50, 61 (1977).
- 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"  
Chem. Phys. Letters, 51, 13 (1977).
- R. H. Clarke and D. R. Hobart  
"Structural Aspects of the Reaction Center of Photosynthetic Bacteria Calculated from Triplet State Zero-Field Splittings"  
FEBS Letters, 82, 155 (1977).
- R. H. Clarke, D. R. Hobart and W. R. Leenstra  
"Investigation of the Structure of the Reaction Center Chlorophyll Complex by Optical Detection of Triplet State Magnetic Resonance"  
Semicond. Insul., 4, 313 (1978).
- R. H. Clarke, W. R. Leenstra and W. G. Haggar  
"Observation of a Triplet State in Chlorophyll Protein 668 via Optically Detected Magnetic Resonance"  
FEBS Letters, 99, 207 (1979).

- R. H. Clarke, D. R. Hobart and W. R. Leenstra  
"The Triplet State of the Chlorophyll Dimer"  
J. Am. Chem. Soc., 101, 2416 (1979).
- R. P. H. Kooyman, T. J. Schaafsma, G. Jansen, R. H. Clarke, D. R. Hobart and W. R. Leenstra  
"A Comparative Study of Dimerization of Chlorophylls and Pheophytins by  
Fluorescence and ODMR"  
Chem. Phys. Letters, 68, 65 (1979).

Publications in press

- R. H. Clarke, S. P. Jagannathan and W. R. Leenstra  
"Laser-Microwave Double Resonance Spectroscopy of In Vivo Chlorophyll" in  
Lasers in Photomedicine and Photobiology, R. Pratesi, ed., (Springer-Verlag,  
Berlin, 1980) in press.
- R. H. Clarke  
"The Chlorophyll Triplet State and the Structure of Chlorophyll Aggregates" in  
Light Reactions in Photosynthesis, F. Fong, ed., (Springer-Verlag, Berlin,  
1980) in press.

Publications submitted

- R. H. Clarke, S. P. Jagannathan and W. R. Leenstra  
"Optical-Microwave Double Resonance Spectroscopy of In Vivo Chlorophyll"  
Photochem. Photobiol., submitted.
- R. H. Clarke, ed.  
ODMR Spectroscopy with Biophysical Applications  
(Wiley-Interscience, New York, NY) in preparation for publication in  
September, 1980.

Talks given at major meetings on research under DOE support (5/1/79 - present)

- Third DOE Solar Photochemistry Research Conference, Asilomar, California, June 20-22,  
1979.  
"Structural Features of Chlorophyll Complexes In Vitro and In Vivo As Determined  
by Triplet State ODMR"
- Europhysics Conference on Lasers in Photomedicine and Photobiology, Florence, Italy,  
September 3-7, 1979.  
"Structural Features of Chlorophyll Complexes In Vitro and In Vivo As Determined  
by Laser-Microwave Double Resonance"  
(invited talk).

Graduate theses completed under DOE support (5/1/79 - present)

David R. Hobart, Ph.D., January, 1980

Title of Dissertation: "Triplet State of the Chlorophyll Dimer"

David Michaels, M.S., January, 1980

Title of Dissertation: "ODMR Spectroscopy of Chlorophyll-Protein CP 668"

V. Budget (5/1/79 - 4/30/80)

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, 1980.