Progress Report

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Antenna Organization in Green Photosynthetic Bacteria
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Introduction

This project is concerned with the structure and function of the unique antenna system found in the green photosynthetic bacteria. The antenna system in these organisms is contained within a vesicle known as a chlorosome, which is attached to the cytoplasmic side of the cell membrane. Additional antenna pigments and reaction centers are contained in integral membrane proteins. Energy absorbed by the bacteriochlorophyll c (BChl c) pigments in the chlorosome is transferred via a "baseplate" array of BChl a antenna pigments into the membrane and to the reaction center. A schematic model of chlorosome structure is shown in Fig. 1. This system is similar in some respects to the phycobilisome antenna system found in cyanobacteria and some types of algae, in that a membrane-associated structure absorbs light and transfers it to the membrane where conversion to chemical energy takes place. However, the overall structure, the type of pigments utilized and the nature of the proteins in these two types of membrane-associated antenna bodies are entirely different, and they clearly represent two independent evolutionary solutions to the problem of light collection and excitation transfer.

Personnel and equipment

The past year has been a productive one for this project. We have many new experimental results and a long list of proposed work for next year. The project has benefitted from the efforts of Dr. Dan Brune, a postdoctoral fellow, and George King, a graduate student. We have also had a very productive collaboration with Prof. Robert Knox at the University of Rochester. His student Bruce Wittmershaus has recently joined my group as a postdoc and will continue some of the streak camera experiments described below as one of several projects that he is pursuing.

We have constructed a sensitive and versatile spectrophotometer for measurement of fluorescence, absorption, circular and linear dichroism that is optimized for work in the near infrared. This spectral region is inaccessible with commercial fluorescence instruments. Our instrument can record corrected fluorescence excitation and emission spectra, as well as fluorescence polarization and can also measure absorption spectra of highly scattering samples using an integrating sphere. This instrument is ideal for measurements of fluorescence quantum yields and the efficiency of excitation transfer in a wide range of samples. A proposal for a picosecond fluorescence lifetime instrument is pending with the DOE University Instrumentation Program.

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Spectroscopic studies of BChl c aggregates as a model for chlorosomes

One of the most interesting aspects of the chlorosome antenna system is that the absorbance properties of the BChl c *in vivo* are almost precisely mimicked by aggregates of BChl c in nonpolar organic solvents (Fig. 2). We have completed a study of the spectroscopic and photophysical properties of these aggregated pigments in order to gain insight into the organization of pigments in the chlorosome and have a manuscript nearly ready for submission (preprint attached). While additional work is needed to reach a final conclusion, our present results indicate that the *in vivo* organization of the BChl c in chlorosomes is very similar to that found in the aggregate that we have been investigating. The existence of direct pigment-pigment interactions as the principal determinants of the spectroscopic properties of the antenna pigments *in vivo* strongly suggests that chlorosomes employ a different organizational principle from that found in all other photosynthetic antennas investigated to date, in which pigment-protein interactions are of dominant importance.

Kinetics of excitation transfer *in vivo*

We have completed a study of the fluorescence lifetimes of the various emitting pigments in the antenna system of *Chloroflexus aurantiacus*. We have published some of these findings in the Proceedings of the VII International Photosynthesis Congress, and have a manuscript nearly ready for submission (preprint attached). This work has involved two visits to the Center For Fast Kinetics Research (CFKR) in Austin, Texas, as well as some collaborative experiments with M. Thewalt at Simon Fraser Univ. The most striking result of this study is the extremely short excited state lifetime of the BChl c pigments, <30 ps under a variety of conditions. Because this lifetime is beyond the resolution of the single photon counting technique, we have begun a study using streak camera detection, which is capable of higher time resolution. Using these techniques, we have measured a lifetime of 15 ps for the BChl c emission in isolated chlorosomes (Fig. 1). One more series of streak camera experiments is planned in the near future, after which we plan to prepare a manuscript for publication.

Preparative methods

An attractive aspect of the chlorosome antenna system is that it has proved quite amenable to biochemical resolution procedures. It is our goal to be able to separate each of the pigment species from the others in an undamaged state and investigate the spectroscopic parameters of the pigments individually. In the past year we have made important progress toward that goal, although more work of that sort remains to be done. We can now prepare chlorosomes in which the 740 nm absorbing BChl c has been selectively removed, samples in which the carotenoid has been extracted, and samples in which the 795 nm Bchl a has been chemically oxidized. Much of our work in the coming year will be involved with optimizing our preparative methods and will utilize these preparations.

Fig. 1 Schematic model of Chlorosome Structure from Feick and Fuller, Biochem. 23, 3693-3700, (1984).

Fig. 2 Absorption spectra of chlorosomes (solid line), carotenoid depleted chlorosomes (dotted line), BChl c aggregate (dashed line), and BChl c monomer (alternating long and short dashed line).

Fig. 3. Streak camera picosecond fluorescence measurement on chlorosomes. Dashed line, excitation function, solid line, data and theoretical fit with lifetimes of 14 ps and 258 ps. The shorter lifetime is from Bchl c which emits at 750 nm. The longer component is from Bchl a which emits at 800 nm.

Reprints removed.

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