Progress Report

Structure, Function and Reconstitution of Chlorosome Antennas from Green Photosynthetic Bacteria

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Introduction

This project is concerned with the structure and function of the chlorosome antennas found in green photosynthetic bacteria. Chlorosomes are ellipsoidal structures attached to the cytoplasmic side of the inner cell membrane. These antenna complexes provide a very large absorption cross section for light capture. Evidence is overwhelming that the chlorosome represents a very different type of antenna from that found in any other photosynthetic system yet studied. It is now clear that chlorosomes do not contain traditional pigment-proteins, in which the pigments bind to specific sites on proteins. Instead, the chlorosome pigments are organized *in vivo* into pigment oligomers in which direct pigment-pigment interactions are of dominant importance. Our group has used a multidisciplinary approach to investigate this unique system, as well as the complexes that they directly interact with. Our work has included using model systems, numerous types of both steady-state and ultrafast spectroscopy, molecular biology, protein chemistry and X-ray crystallography. Details of our recent results using these approaches are given below and in the references.

Numbers cited in the sections refer to DOE-sponsored publications that are listed below. Only publications dated 2003 or later are included in this report. In addition to the primary literature reports, a comprehensive review of this area of research has been written (2).

Fenna-Matthews-Olson Bacteriochlorophyll *a* Protein (1,7)

A long-term area of investigation in our group is centered on a variety of measurements on the Fenna-Matthews-Olson (FMO) bacteriochlorophyll *a* protein that serves as an interface between the chlorosome and the membrane in the green sulfur bacteria. The structure of this protein is known at high resolution and is without question the best understood chlorophyll-containing antenna complex. In this past year, we have collaborated with Jim Allen from ASU, an X-ray crystallographer, in obtaining a new high-resolution structure of the FMO protein from *Chlorobium tepidum,* shown in Fig. 1. (1) This structure includes additional structural details not observed in previous structures, including a citrate binding site and an interior water channel.

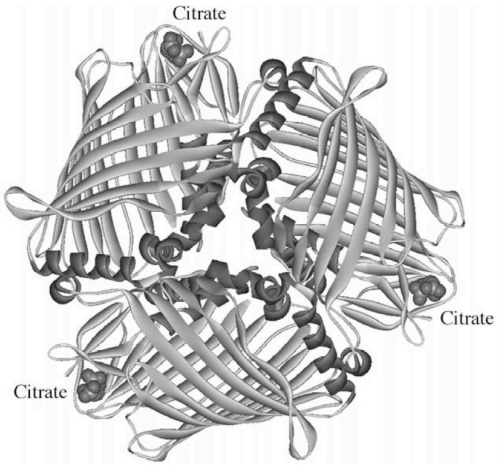

Fig. 1 X-ray structure of FMO protein from *Chlorobium tepidum* at 2.2 Å resolution (1).

We have recently collaborated with Graham Fleming from UC Berkeley in a two dimensional

ultrafast spectroscopic study of the FMO protein from *Chlorobium tepidum* (7). This technique reveals the electronic couplings between the pigments in much the same way that 2D NMR reveals couplings between spins. This work was published in *Nature* and has been widely reported.

Isolation and Characterization of the Chlorosome Baseplate pigment-protein complex (3)

We have successfully isolated and characterized the baseplate complex from chlorosomes of *Chloroflexus aurantiacus* (Fig. 2). This has been a goal of our work for several years and we have finally succeeded in isolating this complex in pure form. It turns out to be what appears to be a new type of pigment-protein complex that contains a single protein (CsmA) and a large amount of carotenoid relative to bacteriochlorophyll. The carotenoid may serve primarily in a structural role, as its energy transfer efficiency to the BChl is not high. This very unusual complex has so far been characterized biochemically and spectroscopically, but not yet structurally. We plan a number of additional studies on it in the near future. This work was initiated by Gabriel Montaño as part of his PhD dissertation research The work is being continued by a new graduate student Amanda Cunow.

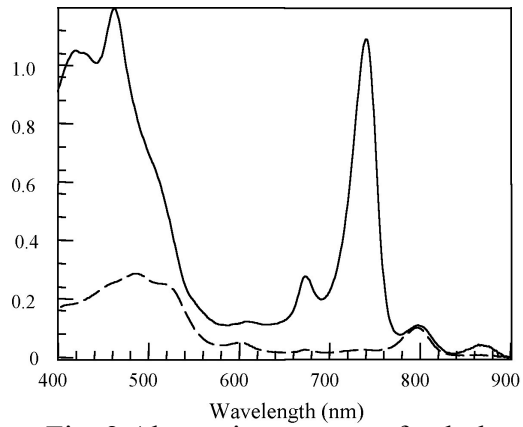

Fig. 2 Absorption spectra of whole chlorosomes (solid) and isolated baseplate (dashed) from *Chloroflexus aurantiacus* (3).

Analysis of Pigment Content of Chlorosomes (4)

For years it has been reported that chlorosomes contain about 10,000 molecules of BChl *c*. However, this number had never actually been determined quantitatively until we undertook a study using fluorescence correlation spectroscopy and dynamic light scattering to resolve this issue. The results were that chlorosomes actually contain significantly more pigments than had previously been thought. Our measurements of *Chlorbium tepidum* indicated that there are actually about 200,000 BChl *c* pigments per chlorosome (4). These results significantly change our view of the numbers of reaction centers that are associated with each chlorosome and correct a long-standing error in the literature. We have not yet successfully extended this approach to *Chloroflexus* chlorosomes due to what appear to be aggregation problems. We are still working on this aspect of the project and plan a new approach using electron microscopy instead of correlation spectroscopy to count chlorosomes. This work was initiated by Gabriel Montaño as part of his PhD dissertation research. The work is being continued by graduate student Martin Hohmann-Marriott.

Isolation and characterization of a FMO-RC Complex

We have devoted a number of years of effort to the biochemical, structural and spectral characterization of the FMO protein, which was the first chlorophyll protein to have its structure determined and still one of the best characterized of all pigment-proteins. However, we still do not understand how the FMO is associated with the reaction center and positioned with respect to the chlorosome. We are now making good progress towards addressing this issue. Our approach is to isolate and characterize an FMO-RC complex. The spectrum of the complex from *Chlorobium tepidum* is shown in Fig. 3. This work is being carried out by an undergraduate Heather Matthies.

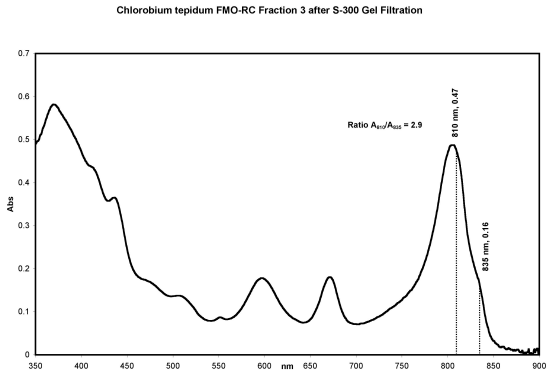

Fig. 3 Absorption spectrum of the FMO-RC complex from *Chlorobium tepidum.*

Spectroscopic and Structural Studies of the B808-866 Integral Membrane Complex from *Chloroflexus auranticacus* (5, 8, 10)

The reaction center in *Chloroflexus aurantiacus* is associated with and almost certainly surrounded by an integral membrane antenna pigment-protein with spectral and sequence properties that clearly shows that it is related to the LH1 and/or LH2 complexes from purple photosynthetic bacteria. This complex is spectrally more similar to LH2 but in terms of sequence and function more like LH1 (Fig. 4). We have isolated this complex both by itself and with associated reaction centers and carried out a number of spectroscopic and microscopic investigations with it. We have measured the energy transfer kinetics both from the carotenoid to BChl and from B808 to B865 Bchls (5, 8, 10). In addition, we have initiated structural studies using atomic force and electron microscopies as well as X-ray crystallography. Our expectation was that there would be a ring with diameter about 110 Å of complexes surrounding the RC in the same way as has been observed for many purple bacteria. However, we were surprised to find significantly larger rings of 200-250 Å diameter. We observed this first using AFM and subsequently confirmed it using TEM as shown in Fig. 5. In addition, we did some dynamic light scattering experiments that gave results completely consistent with this much larger ring. Our current view is that probably multiple (two or three) reaction centers are surrounded by a larger ring of about 20 complexes. This model is consistent with the ring sizes we have observed

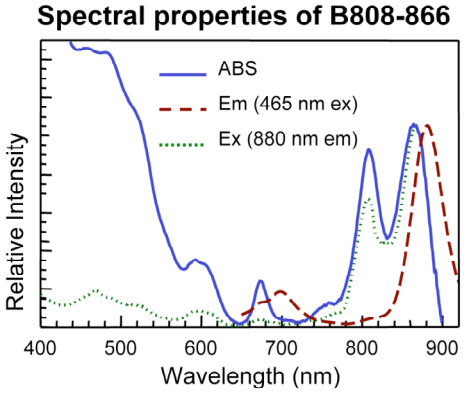

Fig. 4 Spectra of isolated B808-866 complex from *Chloroflexus*.

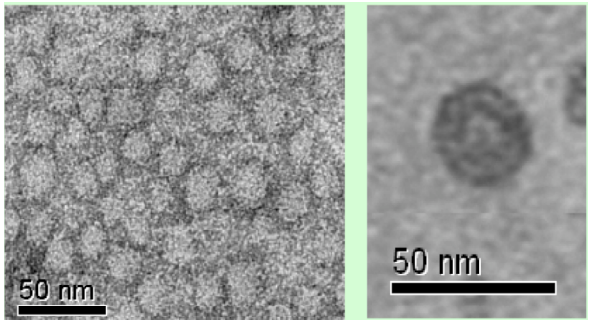

Fig. 5 Transmission electron microscopy images of isolated B808-866 complexes from *Chloroflexus*. Left, aggregated sample; right, diluted sample.

and pigment quantification data that indicates that the ratio of LH complexes to RC is about 12:1 (8, 10). We have very recently obtained crystals of the B808-866 antenna complex that show diffraction to at least 8 Å, which is sufficient to answer the important question of the size of the complex and how many reaction centers are in the center. We have also obtained crystals of the isolated reaction center and preliminary indications are that the complex is dimeric. Although these results are preliminary, we are very excited by them. This work is being carried out by postdoctoral fellow Yueyong Xin.

In a parallel effort, we have cloned the genes for these antenna complexes and sent them to Neil Hunter from the Univ. of Sheffield, who is an expert on both the heterologous expression of antennas in alternate hosts and also in cryo EM studies of LH-RC complexes.

Electron Microscopic Analysis of Green Bacteria (9)

We have nearly completed a complete electron microscopic study of the distribution and biogenesis of chlorosomes in *Chlorobium tepidum* using a range of electron microscopic techniques including tomography. This method reveals the cellular location of chlorosomes and produces a three-dimensional model of the cell (Fig. 6). The first portion of this work is now in press (9) and work for several additional papers is in progress. This work has been carried out by

graduate student Martin Hohmann-Marriott in collaboration with Profs. Robert Roberson of ASU and Richard McIntosh of Univ. Colorado Boulder.

Fig. 6 Three-dimensional model of *Chlorobium tepidum* cell. This is a representation of a dividing cell based on tomograms of three serial sections. Chlorosomes are multicolored and membrane is shown in white outline.

DOE Sponsored Publications 2003-2005

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Published

1. Camara-Artigas A, Blankenship RE and Allen JP (2003) The structure of the FMO protein from *Chlorobium tepidum* at 2.2 Å resolution. *Photosynth. Res.,* 75, 49-55.

2. Blankenship RE and Matsuura K (2003) Antenna complexes from green photosynthetic bacteria. In: *Light-Harvesting Antennas,* BR Green and WW Parson, eds. Kluwer Academic Publisheing (Dordrecht: Kluwer), 195-217.

3. Montaño GA, Wu H-M, Lin S, Brune DC and Blankenship RE (2003) Isolation and characterization of the B798 baseplate light-harvesting complex from the chlorosomes of *Chloroflexus aurantiacus*. *Biochemistry* 42, 10246-10251.

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5. Montaño GA, Xin Y, Lin S and Blankenship RE (2004) Carotenoid and bacteriochlorophyll energy transfer in the B808-866 complex from *Chloroflexus aurantiacus*. *J. Phys. Chem. B* **108**: 10607-10611.

6. Blankenship RE (2004) Identification of a key step in the biosynthetic pathway of bacteriochlorophyll *c* and its implications for other known and unknown green sulfur bacteria. *J. Bacteriol.* **186**: 5187-5188.

7. Brixner T, Stenger J, Vaswani HM, Cho M, Blankenship RE and Fleming GR (2005) Two-dimensional spectroscopy of electronic couplings in photosynthesis *Nature*, **434**, 625-629.

8. Xin Y, Lin S, Montaño GA and Blankenship RE (2005) Structure analysis and excitation transfer dynamics in B808–866 light-harvesting complexes of the green bacterium *Chloroflexus aurantiacus* In: *Photosynthesis: Fundamental Aspects to Global Perspectives*, A van der Est and D Bruce Eds, Vol 1, pps 111-112.

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9. Hohmann-Marriott MF, Blankenship RE, Roberson RW (2005) The ultrastructure of *Chlorobium tepidum* chlorosomes revealed by electron microscopy. *Photosynthesis Research* In press

10. Xin Y, Lin S, Montaño GA, Blankenship RE (2005) Purification and characterization of the B808-866 light-harvesting complexes from green filamentous bacterium *Chloroflexus aurantiacus*. *Photosynthesis Research* In press

11. Beatty JT, Overmann J, Lince MT, Manske AK, Lang AS, Blankenship RE, Van Dover CL, Martinson TA, and Plumley FG (2005) An obligately photosynthetic bacterial anaerobe from a deep-sea hydrothermal vent. *Proc. Natl. Acad. Sci. USA* In press.

12. Hohmann-Marriott MF, Blankenship RE, Sharp W and Roberson RW (2005) Digital position determination system for electron microscopy. *Microscopy Research and Technique*, In Press.