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Determination of the number of bacteriochlorophyll molecules per chlorosome light-harvesting complex in *Chlorobium tepidum*

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Introduction

Chlorosomes are ellipsoidal light-harvesting complexes present in green bacteria(Blankenship et al., 1995). They are attached to the inner membrane of the cell and transfer energy to the membrane bound reaction centers (Blankenship et al., 1995). Chlorosome size varies, depending on the bacterium. *Chlorobium tepidum* contain chlorosomes on the order of 140x50x20 nm and are attached to the membrane via the Fenna-Matthews-Olson pigment-protein (Oelze and Golecki, 1995). Chlorosomes contain pigments mainly consisting of bchl *c, d, or e* with a small amount of Bchl *a* and a varying amount of protein depending on the species.

The bchl *c*(*d,* or *e*) present in the chlorosome is believed to organize into rod-like aggregates which fill the interior of the chlorosome(Blankenship et al., 1995, Staehelin et al., 1978, Staehelin et al., 1980). Over the years, many aggregate models have been proposed based on exciton dynamics within the chlorosomes and in vitro aggregate model studies(Blankenship et al., 1995). Results have varied as to pigment-pigment interaction as well as aggregate-aggregate interaction and interaction with other chlorosomal components. Numbers as to how many bchl *c* molecules per chlorosome have also varied over the years from a few thousand(Olson, 1998) to tens of thousands(Prokhorenko et al., 2000). However, this number can vary greatly depending on the model of aggregation accepted and overall geometry of the chlorosome.

These studies were performed to directly determine the amount of bchl *c* in a typical chlorosome. Chlorosomes from *Chlorobium tepidum* were isolated and examined using fluorescence correlation spectroscopy (FCS), dynamic light scattering (DLS) and biochemical pigment analysis. Results have been compared to existing models of bchl *c* aggregation and conclusions drawn.

Materials and Methods

Chlorobium tepidum was grown phototrophically at 40°C. The cells were harvested by centrifugation at 10,000 x g for 10 minutes after 3-4 days of growth for immediate use. Chlorosomes were then isolated by a modification of methods described by Feick and Fuller(1984). Briefly, cells were resuspended in 0.13% miranol: 10mM Tris-HCl: 10mM

ascorbic acid, homogenized and broken in a French Press at 20,000 psi. The membranes and cell debris were then pelleted by centrifugation at 12,500 x g for 20 min and the supernatant liquid loaded onto 10-40% sucrose gradients containing 0.13% miranol. The gradients were centrifuged at 266,000 x g for 18 hours and the chlorosomes collected from a band in the gradient located between 25-30% sucrose. Sucrose gradients were repeated, and isolated chlorosomes dialyzed in 10mM Tris-HCL buffer pH 8.0 containing 0.13% miranol.

Pigment extractions were performed by adding a small volume of isolated chlorosome sample to methanol and absorbance spectra taken immediately. Absorbance spectra were taken using a Shimadzu UV-2501PC spectrophotometer with a 2 nm spectral band width. bchl *c* absorbance was monitored at 670 nm and an extinction coefficient of 74 mM-1 used(Feick and Fuller, 1982).

Dynamic Light Scatter studies were performed using the DynaPro MS/X (Protein Solutions Inc). Analysis was performed utilizing the DYNAMICS V6 software, and all experiments were run at an operating temperature of 25 °C. All samples were prepared by syringe filtering 20 µl of chlorosome solution after the second sucrose gradient ultra-centrifugation into a quartz cuvette. A laser intensity of 10% was used, with 2 acquisitions per second, and a sample set of >20 acquisitions. Raw data was filtered to remove “dust events” by ignoring data with Sum of Squares (SOS) error fluctuation > 50%. A polydisperse model was used to determine a 95.6% (by mass) hydrodynamic radius (R_h).

For the fluorescence correlation spectroscopy experiments, a frequency doubled, pulse compressed, and mode locked Nd:YAG laser (532 nm, 10 psec) was used to excite the sample at a repetition rate of 82 MHz. To ensure proper beam quality and polarization, the light was passed through a single mode, polarization preserving glass fiber (F-SPA, Newport, Irvine, CA) and a polarizing beam splitter (05BC15PH.3, Newport, Irvine, CA). The laser light was delivered into an inverted, confocal microscope and reflected up towards the microscope objective with a dichroic mirror (Q570LP, Omega Optical, Brattleboro, VT). The sample, a 50 microliter droplet, was spread onto a glass cover slip (22x50mm No 1.5, VWR, West Chester, PA). The same objective (100x PlanApo 1.4NA, Olympus, Tokyo Japan) used to focus the laser also collected the fluorescence. The collected fluorescence passed through the dichroic mirror and was focused onto a 50 micron diameter pinhole (910-PH50, Newport, Irvine, CA). A 720 nm longpass filter was used to separate fluorescence resulting from chlorosome excitation from scattered light. The fluorescence was then split by a polarizing beam splitter (05FC16PB.3, Newport, Irvine, CA) sending photons polarized parallel to the laser to detector one (Perkin Elmer, SPCM-AQR-12, Canada) and photons polarized perpendicular to the laser to detector two. The signal from one of the two detectors was sent to a digital correlator (Flex2K-12x2, correlator.com, USA) that reports the fluorescence autocorrelation function to the computer.

OD670	Bchl *c* (conc.)	Chlor. Conc.	Rh(DLS)	Rh(FCS)	Bchl *c*/ Chlorosome
		2.6×10^{-9} ±1.2×10^{-9} M	26.8± 3.7 nm		
42.1	5.7×10^{-4} ±7.0×10^{-5} M			25.2 ± 3.2 nm	215,000 ± 80,000

Results

With a sample of chlorosomes with OD_{740} ~43, the concentration of bchl *c* was determined to be $5.7 \times 10^{-4} \pm 7.0 \times 10^{-5}$M. Using fluorescence correlation spectroscopy, the concentration of chlorosomes in this same sample was found to be $2.6 \times 10^{-9} \pm 1.2 \times 10^{-9}$M. The model shown in the inset of figure 1 accounts for the correlation of fluorescent events in terms of their diffusivity (D), the average number of molecules in the probe volume (N), the ratio of background intensity to signal intensity (I_b/I_s), and the dimensions of the probe volume (Zander et al., 1996). A hydrodynamic radius of 25.2 +/- 3.2 nm was calculated from the diffusivity according to the Stokes-Einstein equation. A probe volume of 1.6 +/- 0.75 fL was measured for this system having $1/e^2$ radius ω_o=330 +/- 0.20 nm and zo=7.6 +/- 2.6 μm. Dynamic light scattering was performed on the same chlorosome sample to determine the hydrodynamic radius by a different method. The sample was found to have a similar hydrodynamic radius of 26.8 ±3.7 nm (figure 2). Using the numbers obtained for chlorosome and bchl *c* concentration, *Chlorobium tepidum* chlorosomes were determined to contain 215,000± 80,000 Bchl *c* per chlorosome. The hydrodynamic radii values obtained from both types of measurements also fit well with estimated sizes of *Chlorobium tepidum* chlorosomes. Determination of bchl *c*/chlorosome and reported concentrations can be seen in table 1 above.

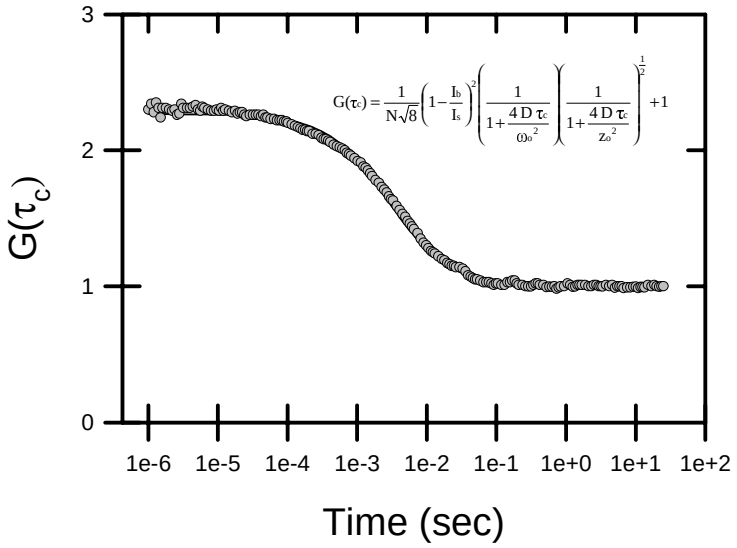

Figure 1- Fluorescence autocorrelation function curve for 2.1×10^{-10}M *Cb. tepidum* chlorosomes having a hydrodynamic radius of 24 nm. The raw data was fit to the model shown in the inset of this plot. This model has been described in previous literature. (Zander et al., 1996)

Discussion

The results presented in this study give the first direct determination of chlorosome pigment concentration. The hydrodynamic radius determined for the chlorosomes fits well with previously reported dimensions for *Chlorobium tepidum* chlorosomes. The consistency of the hydrodynamic radius obtained by both DLS and FCS measurements also supports the accuracy of these values.

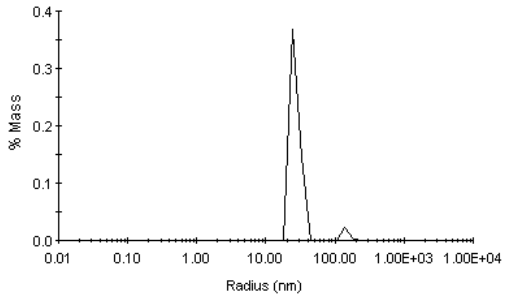

Figure 2-Plot of hydrodynamic radius of chlorosomes as taken by dynamic light scattering. Chlorosomes found to be 26.8 ± 3.7 nm.

Also, the amount of bchl *c* per chlorosome fits well with proposed models for rod element structure. Using theories for rod element structure (Prokhorekno et al., 2000), a number of ~15,000 bchl *c* per Chlorobium type rod element can be determined. Fitting this number to our bchl *c* concentration would give chlorosomes which contain ~14 rod elements. The hydrodynamic radius for an object containing the volume of 14 such rod elements gives a hydrodynamic radius of ~36 nm. This is within the numbers presented here. This is also within numbers determined from previous EM studies (Staehelin, et al., 1980). These findings differ from previous reported numbers, however do support current rod element models. Similar studies performed on *Chloroflexus aurantiacus* yielded vastly different results presumably due to perceived chlorosome aggregation. Methods to disassociate the chlorosomes are currently being looked into in order to determine bchl *c* concentration in these chlorosomes as well.

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