Isolation and Characterization of the B798 Light-Harvesting Baseplate from the Chlorosomes of Chloroflexus aurantiacus †

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Isolation and Characterization of the B798 Light-Harvesting Baseplate from the Chlorosomes of *Chloroflexus aurantiacus*†

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ABSTRACT: The B798 light-harvesting baseplate of the chlorosome antenna complex of the thermophilic, filamentous anoxygenic phototrophic bacterium *Chloroflexus aurantiacus* has been isolated and characterized. Isolation was performed by using a hexanol-detergent treatment of freeze−thawed chlorosomes. The isolated baseplate consists of Bchl *a*, *β*-carotene, and the 5.7 kDa CsmA protein with a ratio of 1.0 CsmA protein/1.6 Bchl *a*/4.2 *β*-carotenes. The baseplate has characteristic absorbance at 798 nm as well as carotenoid absorbance maxima at 519, 489, and 462 nm. The energy transfer efficiency from the carotenoids to the Bchl *a* is 30% as measured by steady-state and ultrafast time-resolved fluorescence and absorption spectroscopies. Energy equilibration within the Bchl *a* absorbing regions exhibits ultrafast kinetics. Circular dichroism spectroscopy shows no evidence for excitonically coupled Bchl *a* pools within the 798 nm region.

The green photosynthetic bacteria are anoxygenic phototrophs that contain an antenna structure known as the chlorosome (*1*, *2*). Chlorosomes transfer energy to the photosynthetic reaction centers. Both the green sulfur and filamentous anoxygenic phototrophic (FAP)[1] bacteria (*3*) contain chlorosomes with similar architecture; however, the pigment content and overall makeup of the chlorosome are dependent on the species (*1*). Green sulfur bacterial chlorosomes are attached to Fenna−Matthews−Olson (FMO) antenna proteins that serve as an intermediary in energy transfer to the photosynthetic reaction centers (*1*, *2*). FAP bacteria lack the FMO complex, and the chlorosomes instead are attached directly to the integral membrane photosynthetic complexes (*1*). The chlorosomes of the FAP bacterium *Chloroflexus aurantiacus* (150 × 50 × 10 nm) sit on the cytoplasmic side of the membrane, efficiently absorb light energy, and funnel it to the reaction center via the B808−866 integral membrane antenna complex (*1*, *4*, *5*).

The chlorosome is an unusual antenna complex in that it contains a very high pigment-to-protein ratio and is believed to rely primarily on pigment−pigment interactions instead of pigment−protein interactions (*1*). Chlorosomes contain a large amount of bacteriochlorophyll *c*, *d*, or *e*, carotenoids, and a small amount of Bchl *a* along with proteins. The complex is surrounded by a monolayer membrane composed primarily of monogalactosyl diglyceride (MGDG) (*1*, *6*, *7*).

Pigment−pigment aggregation in the chlorosome of *C. aurantiacus* causes a spectral shift from 670 nm (monomeric Bchl *c*) to 742 nm (aggregated Bchl *c*) (*1*). The aggregates organize themselves into rod-shaped structures that fill the interior of the chlorosome (*4*, *8*) and pass energy to the B798 nm baseplate (*6*, *7*) that serves as an intermediary in energy transfer to the membrane antenna and reaction center. The baseplate is believed to be a pigment−protein complex that is situated at the base of the chlorosome and connects it to the membrane-associated pigment−protein complexes (*1*, *7*). Electron microscopy has shown a crystalline structure on the order of 6 nm periodicity that creates the attachment site for the chlorosome to the membrane (*4*). This may be due to the so-called B798 nm baseplate complex, although this has not been demonstrated.

The location and function of the chlorosomal proteins is still under debate. Purified *C. aurantiacus* chlorosomes contain three major protein components—CsmA, CsmM and CsmN—of molecular masses 5.7, 11, and 18 kDa, respectively, and a minor component of mass 5.8 kDa (*1*, *7*). The first three proteins have been localized to the lipid envelope by gold labeling electron microscopy (*9*); however, their specific functional roles have not been determined. None of the proteins were found to be specifically localized to the cytoplasmic membrane side of the chlorosome where the baseplate is presumably located (*9*) using antibody methods, although fractions of the CsmM and CsmN peptides were observed in this region.

Proteolytic digestion experiments and CD spectroscopy have suggested the involvement of proteins in determining the overall chlorosome structure, specifically implicating the 5.7 kDa CsmA protein in the organization of the Bchl *c* aggregates (*10*−*13*). However, the interpretation that the 5.7

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[1] Abbreviations: Bchl, bacteriochlorophyll; MGDG, monogalactosyl diglyceride; SPC, single photon counting; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; FAP, filamentous anoxygenic phototroph.

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kDa CsmA protein is a Bchl *c* binding protein (*9*, *14*) is now considered unlikely. While proteinase K treatment on chlorosomes causes spectral changes in the absorbance of Bchl *c*, it is not clear that this is due to the loss of the 5.7 kDa protein, since proteinase K effectively rids the chlorosome of almost all of its protein content. Also, the loss of the 5.7 kDa protein is accompanied by changes in baseplate absorption, including a blue shift in the Bchl *a* absorption (*11*, *13*).

Other studies have shown a correlation between the inhibition of carotenoid synthesis and levels of CsmA protein in *C. aurantiacus* chlorosomes (*15*, *16*). A similar observation was made with *Chlorobium phaeobacteroides* chlorosomes (*17*). Furthermore, the only spectral changes in these chlorosomes occurred in the respective baseplate absorbing regions (*15*−*17*). Therefore, loss of carotenoids could result in or coincide with loss of the CsmA protein and result in the presence of monomeric Bchl *a* absorption at ∼770 nm and thus the observed baseplate spectral changes.

Recent evidence indicates that the CsmA protein binds Bchl *a* and carotenoids in *Cb. tepidum* (*18*, *19*). However, direct evidence of which protein(s) comprise the chlorosome baseplate in *C. aurantiacus* and its pigment composition has not yet been achieved. Correlations between baseplate absorption and protein concentrations have been made over the years, however, with different results and interpretations (*5*, *7*, *20*). Feick and Fuller (1984) proposed that the minor 5.8 kDa peptide served as the Bchl *a* binding protein. Other groups have argued against this interpretation, finding no correlation between Bchl *a* 798 nm absorption and the amount of 5.8 kDa peptide present (*11*, *21*).

C. aurantiacus also contains a membrane bound B808−866 light-harvesting complex, which according to sequence homology is related to the LH1 complexes from purple photosynthetic bacteria (*22*−*24*). However, this complex is more similar spectrally to the purple bacterial LH2 complexes with two pronounced peaks in the infrared due to Bchl *a* absorption (*7*).

Recent evidence pointed to the association of the 5.7 kDa protein, now known as the CsmA protein (*12*), as the baseplate complex protein. The 5.7 kDa protein has been shown to be associated with baseplate absorption (*25*). Also, its degradation is correlated with baseplate degradation (*11*, *13*). These findings have altered the previously accepted view that CsmA binds Bchl *c*.

We used a modification of the procedure of Sakuragi et al. (*25*) to successfully isolate the baseplate and have biochemically and spectroscopically characterized it. This method relies on the disruption of chlorosomal organization from exposure to freezing temperatures, using a hexanol treatment on isolated chlorosomes (*26*, *27*) and detergent extraction. We conclude that the baseplate consists of Bchl *a*, *β*-carotene, and the 5.7 kDa CsmA protein. It contains a large amount of *β*-carotene, whose role may be both structural and functional in either light-harvesting or photoprotection.

EXPERIMENTAL PROCEDURES

Chlorosome Preparation and Baseplate Isolation. C. aurantiacus strain J-10fl was grown photoheterotrophically in 1-L batch cultures at 55° C in medium D as previously described (*28*). The cells were harvested by centrifugation at 10000*g* for 10 min after 3−4 days of growth and stored at −20 °C. Cells were frozen at −20° C for ≥2 months, upon which the stability of the 742 nm chlorosome Bchl *c* rod elements decreased, resulting in a significant amount of monomeric 670 nm Bchl *c* in the absorption spectrum. Chlorosomes were then isolated by a modification of methods described by Gerola and Olson (*20*) and Zhu et al. (*28*). Briefly, *C. aurantiacus* cells were resuspended in a 2 M NaSCN/10 mM phosphate/10 mM ascorbic acid buffer, pH 7.4, homogenized and broken in a French Press at 20 000 psi. The membranes and cell debris were then pelleted by centrifugation at 12500*g* for 20 min and the supernatant liquid was loaded onto 5−40% sucrose gradients. The gradients were centrifuged at 266000*g* for 18 h and the chlorosomes collected from a band in the gradient located between 25 and 30% sucrose. The chlorosomes were further purified by gel filtration chromatography over a Sephacryl S300 column and stored at 4 °C.

The chlorosome baseplate was isolated by a modification of the method described by Sukaragi et al. (*25*). Four milliliters of ∼15 OD total, determined from a combination of 670 and 740 nm peak heights, was added to 32 mL of a saturated hexanol solution in 10 mM phosphate buffer, pH 7.4. Twenty-four milliliters of 20% cholic acid in ddH_2O was added to the solution and the mixture was incubated at 37 °C for 6 h. The solution was centrifuged at 266000*g* for 60 min, and the pellet was collected. The pellet collected was then homogenized and centrifuged at 3000*g*. The supernatant was collected, dialyzed in 10 mM phosphate, pH 7.6, and used in characterization studies of the baseplate as an aggregated hydrophobic protein complex of a large size but homogeneous with regard to composition.

Steady-State Absorption and Fluorescence Spectroscopy. Absorbance spectra were taken using a Shimadzu UV-2501PC spectrophotometer with a 2-nm spectral bandwidth. Steady-state fluorescence spectra were taken using a Photon Technology International (PTI) fluorimeter with an Advanced Photonics Inc. avalanche photodiode detector. Excitation spectra were obtained with emission monitored at 825 nm and emission spectra with excitation at 485 nm. Spectral bandwidth was 4 nm.

SDS−PAGE. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) was performed according to Schägger and Von Jagow (*29*). Five micrograms of protein from chlorosome and baseplate samples was applied to the gel in the presence of a 2% ***β***-mercaptoethanol/100 mM Tris/8% SDS/24% glycerol/0.02% bromophenol blue dissociation buffer, pH− 6.8. This was applied to a 16.5, 10, and 4% acrylamide as separating, spacer, and stacking gel, respectively. After electrophoresis, the gels were stained with Coomassie blue R-250.

Mass Spectrometry. For mass spectrometry, samples were prepared by diluting a small volume of sample in α-cyano-4-hydroxycinnamic acid matrix. Samples were run on a Vestec Lasertech Research MALDI-TOF mass spectrometer operated in the positive ion mode.

Protein Sequencing. A baseplate sample was run on SDS−PAGE as described above and transferred to a polyvinylidene fluoride (PVDF) membrane using a 10 mM 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS) buffer pH 11.0 w/20% methanol. The PVDF membrane was then stained

with Coomassie blue R-250. The CsmA band was cut from the membrane and sequenced using a Porton 2090E protein sequencer (Beckman Coulter, Fullerton, CA). A sequence was also obtained directly from a liquid sample in the same manner as above, yielding the first four amino acids.

Circular Dichroism. For circular dichroism studies, isolated baseplate sample of OD_{798} nm of 0.4 was used. Four spectra were scanned and averaged using a Jasco 710 CD spectrometer with a slit width of 300 μm and a sensitivity of 20 mdeg.

Ultrafast Spectroscopy. One milliliter of isolated baseplate and isolated chlorosome samples $OD_{798} \sim 0.2$ was used in the fluorescence lifetime measurements. Fluorescence lifetimes were measured using a using a single photon counting apparatus previously described (*30*). Excitation of the sample was directly into the Q_x absorption band of the baseplate or chlorosome Bchl *a* at 605 nm and fluorescence emission was detected between 780 and 870 nm.

Femtosecond transient absorption spectroscopy was performed using a pump−probe setup described previously (*31*). The sample was excited at 780 and 810 nm with 100 fs pulses at a repetition rate of 1 kHz. The absorbance changes were recorded between 740 and 910 nm with a 2-nm wavelength resolution. Laser excitation of 2 mW was used and the polarization was set at a magic angle in relative to that of the probe beam. Global analysis was performed on the data to obtain decay-associated spectra.

Baseplate Pigment and Protein Composition Determination. To determine pigment ratios, equal volumes of sample were dried and dissolved into either methanol for Bchl *a* or hexane for β-carotene and absorption spectra obtained. Molar extinction coefficients were then used to calculate the relative molar ratio of pigments. 76 mM^{-1} cm^{-1} was used for Bchl *a* (*32*) and 128 mM^{-1} cm^{-1} for β-carotene (*33*).

For protein composition determination, a measured amount of sample was dried and subjected to vapor phase hydrolysis at 105 °C for 24 h in an evacuated tube containing 200 μL 6 M HCl containing 1% phenol and analyzed by a Hewlett-Packard AminoQuant amino acid analyzer. A 5 μg sample of dried alpha lactalbumin was used as a control. After precolumn derivatization of primary amino acids with *o*-phthalaldehyde and proline with 9-fluorenylmethyl chloroformate, the sample was automatically injected onto a C-18 HPLC column where the amino acids were separated. Amino acids were identified from their retention time on the column and quantified from the areas under their fluorescence peaks after passing through a fluorescence detector.

RESULTS

The chlorosome baseplate was isolated using a hexanol-detergent treatment of chlorosomes obtained from freeze−thawed *C. aurantiacus* cells. The absorption spectrum of the isolated baseplate sample showed a large peak at 798 nm characteristic of the baseplate Bchl *a* and a large carotenoid contribution with maxima at 519, 489, and 462 nm (Figure 1). A minor peak appeared at 670 nm due to residual monomeric Bchl *c*. By both mass spectrometry and SDS−PAGE (Figures 2 and 3), only one protein was present in the baseplate isolate, the 5.7 kDa CsmA protein. Sequencing of the isolated protein also showed the protein to be the 5.7 kDA CsmA protein. The first seven amino acids were

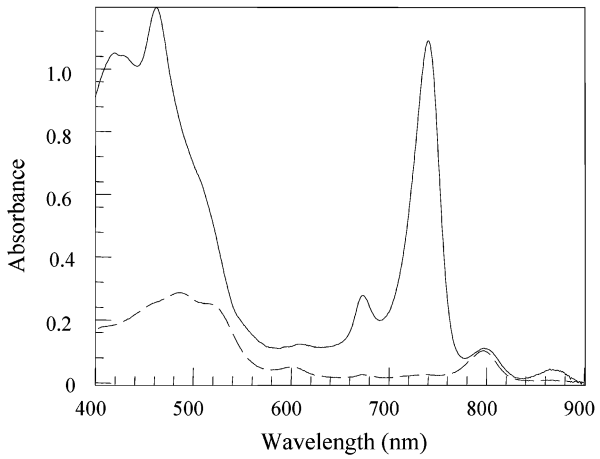

FIGURE 1: Absorption spectra of isolated chlorosomses (solid line) and isolated baseplate (dashed line). The isolated baseplate shows absorbance at 798 nm and a large contribution between 400 and 550 nm dominated by carotenoid absorption. The isolated chlorosome sample shows in addition to the baseplate complex absorption, a large absorption peak at 742 nm due to oligomeric Bchl *c*, a small peak at 670 nm due to monomeric Bchl *c*, and a small peak at 866 nm due to B866 nm absorption.

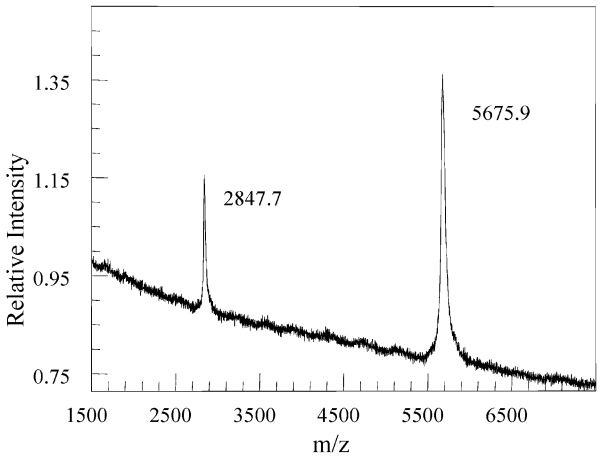

FIGURE 2: MALDI-TOF mass spectrometry of isolated baseplate sample. CsmA mass is 5679 Da. Mass spectrum determined a protein size of 5675.9 Da with the double charged peak at 2847.7 Da. No other proteins were found by mass spectrometry.

sequenced and matched perfectly with the first seven N-terminal amino acids of the CsmA protein (data not shown) (*10*).

The carotenoid baseplate absorbance maxima is red-shifted from those of either β-carotene or γ-carotene in solution, the two principal carotenoids found in *C. aurantiacus* (*34*). Extraction and resolubilization of the carotenoid in acetone showed it to have the spectral profile of β-carotene. Relative pigment and protein concentrations were determined and are given in Table 1. A given volume of baseplate sample was also used to determine protein concentration by amino acid analysis. The ratios were determined to be 1.6 Bchl *a*/1.0 CsmA/4.3 β-carotene.

Steady-state fluorescence excitation spectra, monitored at the Bchl *a* emission at 815 nm, showed energy transfer occurring from the β-carotene to the 798 nm absorbance band of the baseplate with an efficiency of ~30% (Figure 4). This is less than that seen for many other similarly long-chained carotenoids in photosynthetic antenna complexes (*35*) that exhibit energy transfer; however, carotenoid to chlorophyll

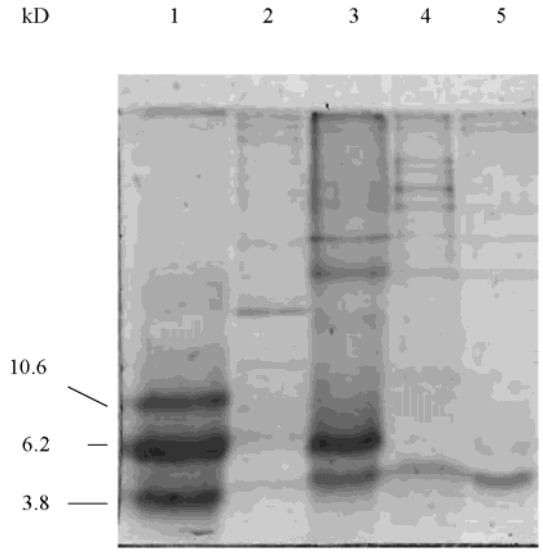

FIGURE 3: SDS−PAGE of isolated chlorosomes and isolated baseplate samples. Lane 1, molecular marker. Lane 2, isolated chlorosomes. Lane 3, isolated *C. aurantiacus* membrane complex. Lane 4, isolated baseplate sample w/small 866 nm shoulder. Lane 5, isolated baseplate sample. Only 1 protein, the 5.7 kDa CsmA protein is present in the isolated baseplate sample.

Table 1: Baseplate Pigment and Protein Concentrations[a]

CsmA	Bchl *a*	β-carotene
	molar concentrations	
8.7×10^{-6} M $\pm 1.2 \times 10^{-6}$	1.43×10^{-5} M $\pm 6.0 \times 10^{-7}$	3.73×10^{-5} M $\pm 7.0 \times 10^{-7}$
beta carotene: Bchl *a*	beta carotene: CsmA	Bchl *a*: CsmA
	relative molar ratios	
2.6:1 ± 0.15	4.3:1 ± 0.70	1.6:1 ± 0.30

[a] Molar ratio determination of isolated baseplate components.

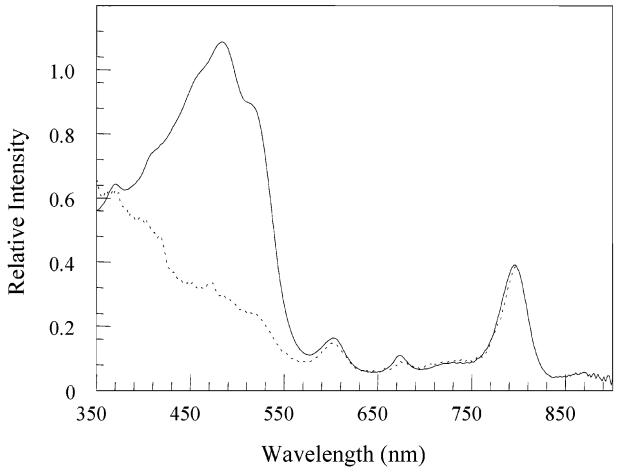

FIGURE 4: Absorbance (solid) and fluorescence excitation (dashed) spectra. For excitation spectrum, emission was monitored at 825 nm and spectra were normalized at 798 nm, the Qy absorption of Bchl *a*.

energy transfer values vary widely. Single photon counting (SPC) fluorescence and pump−probe transient absorption spectroscopy were used to monitor the energy transfer processes within the baseplate. Analysis of the fluorescence kinetics of the baseplate monitored at 810 nm reveals three lifetimes of 40 (59.6%), 140 (36.8%), and 400 (3.6%) ps

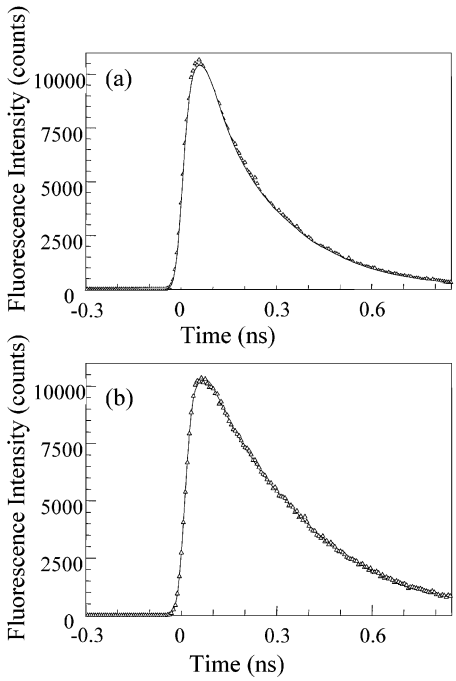

FIGURE 5: Single photon counting fluorescence of isolated baseplate sample (a) and isolated chlorosome sample (b). Baseplate showed three fluorescence decay components: $T_1 = 40$ ps (59.6%), $T_2 = 143$ ps (36.8%), $T_3 = 396$ ps (3.6%). Chlorosomes also had three fluorescence decay components: $T_1 = 35$ ps (52.2%), $T_2 = 199$ ps (3.9), $T_3 = 380$ ps (9.8). Excitation at 605 nm and fluorescence emission monitored at 810 nm.

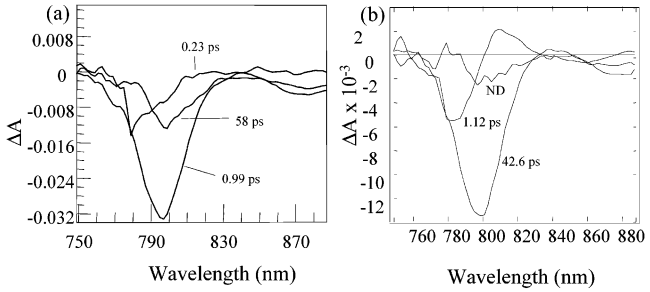

FIGURE 6: Transient absorption spectra and decay associated spectra of isolated baseplate sample with 780 nm excitation. Absorbance changes monitored from 750 to 890 nm. (a) Transient absorption spectrum at given times. (b) Global fitting of transient absorption spectra. Three decay components were obtained: $T_1 = 1.12$ ps, $T_2 = 42.6$ ps, $T_3 =$ ND component.

(Figure 5). Similar kinetic processes were also observed from the isolated chlorosome samples, excited directly into the baseplate at 605 nm.

Transient absorption spectroscopy on baseplate samples, with 780 nm excitation, showed rapid relaxation of the excitation energy to ~800 nm, within 1−2 ps, followed by excited-state decay kinetics similar to that observed with SPC (Figure 6a). Global analysis of the baseplate transient absorption data returned three kinetic components of 1.1, 43, and a ND component (Figure 6b). The derivative shape of the 1.1 ps decay associated spectrum is due to excitation redistribution within the baseplate. The latter two lifetimes are similar to those obtained from fluorescence measurements, showing decay processes of the excited state.

The CD spectrum shows no derivative shaped signal in the B798 region characteristic of exciton coupling (Figure 7). There is, however, a large negative dichroism centered

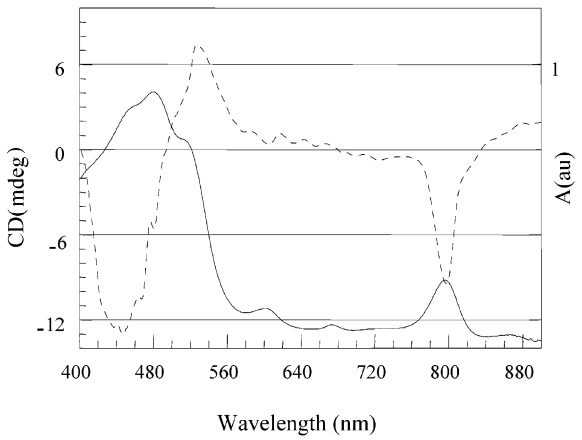

FIGURE 7: CD absorption spectrum of isolated baseplate sample. Absorbance spectrum (solid line) and circular dichroism spectrum (dashed line) of isolated baseplate complex.

around the B798 nm absorption, as well as a strong signal in the carotenoid absorption region.

DISCUSSION

The experiments performed in this study have led to the isolation and initial characterization of the B798 light-harvesting baseplate from *C. aurantiacus.* The characterization of the baseplate indicates a new type of pigment−protein organization and a new type of light-harvesting structure. By using a hexanol-detergent treatment of disrupted cells, it was possible to obtain a sample of baseplate that contained only one protein, the 5.7 kDa CsmA protein, Bchl *a*, and β-carotene. A small peak observed in the absorption spectrum at 670 nm is attributed to a trace of residual Bchl *c*.

The presence of the 5.7 kDa CsmA protein as the Bchl *a* baseplate binding peptide supports recent evidence that the baseplate is a pigment−protein rather than a pigment oligomer (*36*), and direct evidence associating the CsmA protein with the baseplate (*25*). The presence of only this protein in the sample along with the large amount of pigment found in the sample indicates a possible new type of light-harvesting protein complex. The large amount of β-carotene present in the sample was surprising because the majority of carotenoid in the chlorosome has previously been thought to be present in the body of the chlorosome, and to transfer energy to the Bchl *c*, rather than to the Bchl *a* (*1*, *5*). However, energy transfer efficiencies from fluorescence excitation studies may have been previously misinterpreted, with the Soret contribution of Bchl *c* being mistaken for carotenoid (*5*). The β-carotene could also prove to have more than one location within the chlorosome. The excitation spectrum shows that there is energy transfer to the B798 nm Bchl *a* of the baseplate from the β-carotene of ~30%. However, it remains to be seen whether there is also a structural role for the β-carotene or a photoprotection role. The large amount of carotenoid relative to the Bchl *a* suggests that one or both of these may indeed be the case. Also, the baseplate receives energy from the bulky chlorosome which contains tens of thousands of Bchl *c* molecules, making the need for photoprotection likely to be more important than antenna function. Carotenoids have also been found to serve structural roles in other light-harvesting complexes such as the LH2 of purple bacteria and the peridinin−chlorophyll protein (*37*, *38*). The peridinin−chlorophyll *a* protein complex also contains a large amount of carotenoid relative to Bchl *a* and has an absorption spectrum in which the largest spectral contribution arises from the peridinin carotenoids (*38*, *39*).

The kinetics of the baseplate excited states obtained from ultrafast time-resolved measurements are generally consistent with those of other studies on whole chlorosomes. Single photon counting fluorescence exhibited the typical decay components that have previously been associated with the baseplate. However, the main component was found to be the 40 ps component, whereas other studies have shown the major component to be anywhere from 100 to 400 ps (*40*, *41*). The 40 ps component was previously thought to be due to energy transfer into the B808−866 light-harvesting complex. The 150−200 ps component has been noted as possibly being due to energy transfer within the baseplate to a location where B808−866 is attached (*41*). This component, which is seen as the major component in other studies (*40*−*42*), is a smaller contributor in our studies, both with whole chlorosomes and isolated baseplates. The long-lived 350−400 ps component has been previously reported as due to the decay of the Bchl *a* (*41*), and has been reported as a minor component as it is in our studies.

The ultrafast transient absorption studies corroborated the SPC results. On a short time-scale there appeared to be no evidence for separate pools of Bchl *a* within the B798 region. Excitation at either 780 or 810 nm resulted in relaxation to 795−800 nm within 2 ps. A weak bleaching in the 860−880 nm region was also observed within 2 ps, probably due to the direct excitation of some remaining B808−866 complexes in the sample. The residual B808−866 complexes cannot be responsible for the 40 ps excitation trapping judging from the amount of bleaching formed (less than 10%) and its formation time. The 40 ps lifetime is the dominant process of intrinsic excitation decay of the isolated baseplate. Furthermore, solubilization of isolated baseplate complex with a large amount of 866 nm absorbance in 1.0% β-OG followed by centrifugation at 18000*g* results in a pellet which was determined to have the 4.8 and 6.2 kDa peptides of the B808−866 complex by mass spectrometry. This was previously undetected. Therefore, the possibility that any of the absorbance at 866 nm is due to an intrinsic long wavelength component of the baseplate, as was reported in a preliminary communication (*43*), now appears unlikely.

This study reports the first isolation of the minimal B798 baseplate from *C. aurantiacus*. It has shown to consist of CsmA protein, Bchl *a*, and β-carotene. The isolation of the baseplate has now raised several important issues. To understand the physical makeup of it, structural determination must be completed. It has been shown to contain a large amount of carotenoid and contains only the 5.7 kDa CsmA peptide. The structure may indeed be found to represent a new architecture of light-harvesting complexes.

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