A Reconstituted Light-Harvesting Complex from the Green Sulfur Bacterium *Chlorobium tepidum* Containing CsmA and Bacteriochlorophyll *a*†

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ABSTRACT: Green sulfur bacteria possess two light-harvesting antenna systems, the chlorosome and the Fenna−Matthews−Olson (FMO) protein. In addition to self-aggregated bacteriochlorophyll (BChl) *c*, chlorosomes of *Chlorobium tepidum* contain a small amount of BChl *a* (ratio 100:1). The chlorosomal BChl *a* is associated with CsmA, a 6.2 kDa protein that accounts for more than 50% of the protein content of chlorosomes. This CsmA−BChl *a* complex is located in the chlorosome baseplate with the hydrophilic C-terminal part of CsmA in contact with the FMO protein. CsmA was purified from *Chl. tepidum*. Isolated chlorosomes were lyophilized and extracted with chloroform/methanol (1:1, v/v). The extract was further purified using gel filtration and reverse-phase HPLC and the purity of the preparation confirmed by SDS−PAGE. Mass spectrometric analysis showed an *m*/*z* of 6154.8, in agreement with the calculated mass of the *csmA* gene product after C-terminal processing. CD spectroscopy of the isolated protein showed that the main structural motif was an α-helix. We have reconstituted the isolated CsmA protein with BChl *a* in micelles of *n*-octyl β-D-glucopyranoside. The resulting preparation reproduced the spectral characteristics of the CsmA−BChl *a* complex present in the chlorosome baseplate.

Photosynthetic organisms have evolved a wide variety of light-harvesting antenna systems with different accessory pigments. The largest antenna system currently known is the chlorosome found in two phylogenetically diverse groups of bacteria: the green sulfur bacteria (Chlorobi) and the green filamentous bacteria (Chloroflexi) often represented by *Chlorobium* (*Chl.*[1]) *tepidum* and *Chloroflexus* (*Cfx.*) *aurantiacus*, respectively. Possession of chlorosomes enables these bacteria to live in extremely low light environments, as demonstrated by the finding of green sulfur bacteria in the chemocline of the Black Sea at a depth of 100 m (*1*).

Chlorosomes are elongated bodies appressed to the cytoplasmic surface of the cell membrane, and the ability of the chlorosome to harvest light at extremely low photon flux is the result of a high pigment density. In contrast to other light-harvesting antennas, such as those in phototrophic proteobacteria, cyanobacteria, and higher plants, where the pigments are organized on protein scaffolds, chlorosome pigments (bacteriochlorophylls *c*, *d*, or *e*, depending on the species) are organized in large oligomers resulting from pigment−pigment interactions without the involvement of proteins (*2*).

As well as the chlorosome bacteriochlorophyll (BChl) *c*, *d*, or *e*, chlorosomes contain small amounts of BChl *a*. This was first detected in a BChl *c*-depleted chlorosome fraction from *Chlorobium limicola*, where the Q_y absorption band of BChl *a* was observed at 794 nm (*3*). An absorption band of BChl *a* around 800 nm could also be detected in the spectra of intact chlorosomes at 4 K (*4*). Spectroscopic analysis showed that this pool of BChl *a* serves as an intermediary in the transfer of excitation energy from the chlorosome antenna to the reaction center (*5*).

The first direct evidence for an association of the chlorosome BChl *a* with the major chlorosome protein CsmA came from detergent treatments of *Cfx. aurantiacus* chlorosomes. All chlorosome proteins except CsmA could be extracted selectively without affecting the spectral properties of BChl *a* (*6*). That CsmA is a BChl *a*-binding protein was later confirmed by the isolation of the "minimal B798 baseplate" of *Cfx. aurantiacus*, consisting of the CsmA protein, BChl *a*, and β-carotene (*7*), and from studies on carotenosomes (chlorosomes lacking BChl *c*) of *Chl. tepidum* (*8*).

The *csmA* gene in *Chl. tepidum* encodes a pre-CsmA protein with 79 amino acids that is modified by cleavage of 20 amino acids from the C-terminal to give the 6.2 kDa protein present in chlorosomes (*9*). Analysis of the protein sequence shows that the N-terminus (residues 1−40) is hydrophobic while the C-terminus (residues 41−59) is hydrophilic. The protein has a single conserved histidine

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[1] Abbreviations: BChl, bacteriochlorophyll; CD, circular dichroism; *Cfx*., *Chloroflexus*; *Chl*., *Chlorobium*; FMO, Fenna−Matthews−Olson; HFA, hexafluoroacetone trihydrate; HPLC, high-performance liquid chromatography; LH, light-harvesting complex, MALDI-MS, matrix assisted laser desorption ionization mass spectrometry; OG, *n*-octyl β-D-glucopyranoside; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; TFA, trifluoroacetic acid; TFE, trifluoroethanol.

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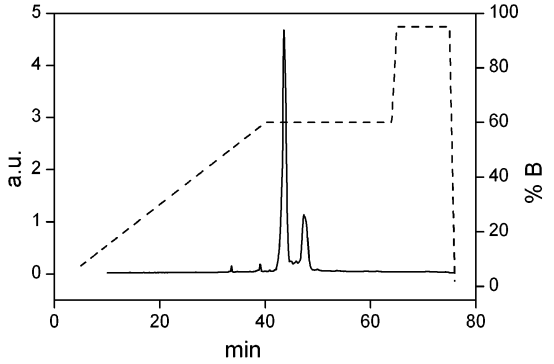

FIGURE 1: HPLC analysis of the combined yellow fractions obtained from a Sephadex LH-20 column. The chromatogram shown (solid line) was recorded at 214 nm. The injection peak has been omitted for clarity. The major peak eluting at 43 min was collected and used for further analysis. The gradient of solvent B is shown in the figure (dashed line). Solvent A, 1:9 (v/v) acetonitrile/water containing 0.1% trifluoroacetic acid; solvent B, 9:1 (v/v) acetonitrile/water with 0.1% trifluoroacetic acid.

(His25) that most likely binds BChl *a*. The results of protease digestion suggest that the hydrophobic part of the protein is embedded in the lipid monolayer that surrounds the chlorosome (*10*). We have recently shown by surface plasmon resonance that in chlorosomes from *Chl. tepidum*, the C-terminal, hydrophilic part of CsmA interacts with the FMO protein (*11*).

In the present study, we have purified and characterized CsmA from *Chl. tepidum* chlorosomes and reconstituted the protein with BChl *a*. The reconstituted preparation reproduced the absorption and CD of the CsmA−BChl *a* complex present in the chlorosome baseplate.

MATERIALS AND METHODS

Isolation of Chlorosomes. Chl. tepidum was grown in continuous culture as previous described (*12*). Chlorosomes were prepared by two consecutive sucrose density gradient centrifugations as described by Milks et al. (*10*). The chlorosomes were dialyzed against 50 mM Tris-HCl (pH 8.0) and freeze-dried before storage at −20 °C.

Purification of CsmA Protein. Freeze-dried chlorosomes were extracted in 1:1 (v/v) chloroform/methanol containing 0.1 M ammonium acetate. The chlorosome extract was loaded onto a Sephadex LH-20 column (20 × 2.5 cm) and eluted with the same solvent using a flow of 1.0 mL/min. CsmA appeared in the first yellow fractions eluted from the column (eluting time approximately 25 min). These fractions were pooled, lyophilized, and redissolved in hexafluoroacetone (HFA) prior to further purification by reverse phase HPLC using a 250 × 10 mm Jupiter C-18 column (5 μm particles, 300 Å pore size) obtained from Phenomenex, Torrance, CA, with a flow rate of 3.5 mL/min and a column temperature of 50 °C. The HPLC solvents were 1:9 (v/v) acetonitrile/water containing 0.1% trifluoroacetic (TFA) (solvent A) and 9:1 (v/v) acetonitrile/water with 0.1% TFA (solvent B). A typical eluting profile is shown in Figure 1.

Isolation of Pigments. BChl *a* was isolated from *Rhodobacter sphaeroides* R 26.2, a carotenoid-less mutant, and BChl *c* was isolated from *Chl. tepidum*. Both pigments were extracted using acetone/methanol 7:2 (v/v) and purified by reverse-phase HPLC on a Novapak C18 column (4 μm particle size, 60 Å pore size, 300 × 3.9 mm) obtained from Waters (Milford, MA) using a linear gradient from 30 to 100% 50:20:30 (v/v/v) methanol/acetonitrile/ethyl acetate, starting from 42:33:25 (v/v/v) methanol/acetonitrile/water.

BChl *a* concentrations were calculated using an extinction coefficient of 69.3 mM^{-1} cm^{-1} in acetone (*13*).

Reconstitution Assay. Aliquots of 80 μg CsmA in HFA were dried under vacuum in 10 mL glass test tubes to form thin, transparent films. A 200 μL portion of 5% *n*-octyl β-D-glucopyranoside (OG) in 50 mM potassium phosphate buffer (pH 7.4) was added to the CsmA film and the mixture incubated for 0.5 h at 4 °C. The solution was diluted in the same buffer to give a final OG concentration of 0.83%. Concentrated BChl *a* (1−5 mM) in acetone was added to give a final concentration of 2−10 μM. The sample was incubated in the dark for 20 h at 4 °C.

Spectroscopy. Absorption spectra were recorded on a Shimadzu UV-1700 spectrophotometer (Kyoto, Japan).

CD spectra were measured at room temperature on a JASCO J-715 spectropolarimeter (Cremella, Italy). Calibration was done using D-(+)-10-camphorsulfonic acid. Circular quartz cuvettes with light paths of 0.2 or 2 cm were used for measurements in far UV or near IR, respectively. The scanning speed was 20 nm/min, 2 pt/nm, using a bandwidth of 2 nm. All CD spectra (except Figure 6) were corrected by subtraction of the background due to the buffer alone. The percent of α-helix was calculated from the molar ellipticity at 222 nm using $\Delta\epsilon = 0$ as 0% and $\Delta\epsilon = -10$ as 100% α-helix (*14*).

SDS−PAGE Analysis. Purified CsmA was analyzed by Tris-Tricine SDS−PAGE as described by Vassilieva et al. (*15*) using a 15% resolving gel with a 4% stacking gel. The gels were silver-stained as described by Schevenko et al. (*16*). Kaleidoscope and Rainbow markers were obtained from Bio-Rad (Hercules, CA) and Amersham Bioscience (Little Chalfort, UK), respectively.

Mass Spectrometry. MALDI-TOF-MS measurements were performed with an Ultraflex time-of-flight mass spectrometer (Bruker Daltonics, Billerica, MA). G2025A peptide standard (Agilient Technologies, Naerum, Denmark) was used for calibration, and the resulting data were analyzed using GPMAW 5.10 (Lighthouse Data, Odense, Denmark). The CsmA protein was dried and dissolved in 5% formic acid. A small volume (0.5 μL) was placed on the target before mixing with 0.5 μL of matrix [2,5-dihydroxybenzoic acid/2-hydroxy-5-methoxybenzoic acid (9:1 by vol) in 50% acetonitrile containing 0.1% TFA].

RESULTS

Purification of CsmA Protein. CsmA was extracted from chlorosomes of *Chl. tepidum*. After fractionation on a Sephadex LH-20 column, the protein was purified by reverse-phase HPLC (Figure 1). All peaks were analyzed by SDS−PAGE. As shown in Figure 2, it was not possible to detect other proteins than CsmA in the main fraction eluding at 43 min, even after silver staining of overloaded gels. Mass spectrometric analysis of this fraction showed the presence of a single peak at $m/z = 6154.8$ corresponding to the mass of processed CsmA retaining the N-terminal methionine (*17*). Spectroscopic analysis showed that this fraction did not contain any pigments (not shown).

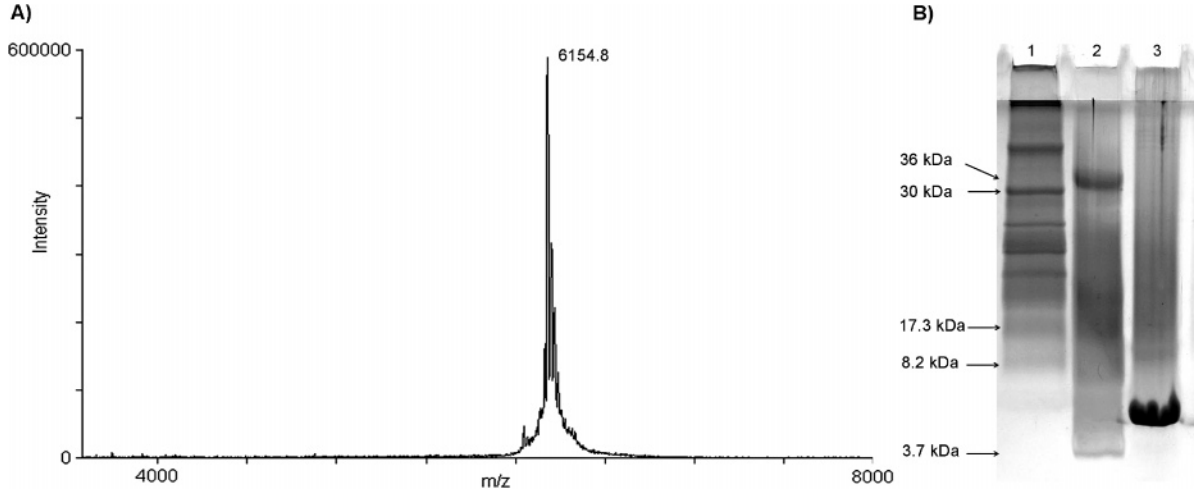

FIGURE 2: Analysis of the major HPLC peak eluting at 43 min (A). MALDI-TOF-MS analysis. The *m*/*z* value given corresponds to the monoisotopic peak (M^+). (B) SDS−PAGE analysis. The gel has been stained with silver. Lane 1, Rainbow marker; lane 2, Kaleidoscope marker; lane 3, sample from HPLC (0.5 mg protein).

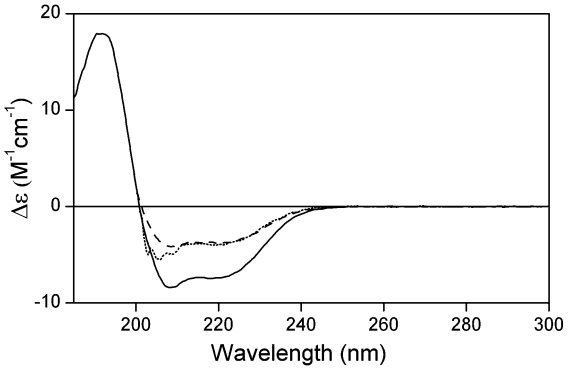

FIGURE 3: Secondary structure of CsmA. A comparison of CD spectra of CsmA in micelles of 0.83% *n*-octyl *β*-D-glucopyranoside in 50 mM potassium phosphate buffer (dashed line) or in hexafluoroacetone trihydrate (dotted line) and trifluoroethanol (solid line). Spectra of CsmA in *n*-octyl *β*-D-glucopyranoside micelles and hexafluoroacetone trihydrate are not showed below 200 nm due to solvent absorption. The concentration of CsmA was in all cases 10 μM.

The purified CsmA was insoluble in aqueous buffers and showed limited solubility in detergent solution and organic solvents. The difficulties found in dissolving CsmA can be explained by the amphiphilic nature of the protein (*10*). The predicted absorptivity of CsmA is 6970 M^{-1} cm^{-1} using GPMAW, and the yield calculated by absorption spectroscopy was in agreement with the weight of product obtained (5 mg from 10 g wet weight of cell pellet) as well as a determination of the protein content by binding of Coomassie Blue (results not shown).

CD Spectroscopy of CsmA. In order to obtain information about the secondary structure of CsmA protein, we recorded CD spectra of the protein in two organic solvents, HFA and trifluoroethanol (TFE), and in micelles of the nonionic detergent *n*-octyl *β*-D-glucopyranoside (OG) (Figure 3). All CD spectra showed typical α-helical patterns. Spectra in TFE showed a negative double peak at 222 and 208 nm and a positive peak around 192 nm. Because of contributions from the buffer in OG or the HFA solvent, the positive peak could only be observed in TFE. The α-helix content was calculated from the ellipticity measured at 222 nm. In OG micelles, the percentage of α-helix was 41% ± 4% ($n = 7$). As seen in Figure 3, the α-helical content of CsmA in HFA (43%) was approximately the same as in OG. In contrast, the α-helix content in TFE was almost doubled to 79%, in agreement with the α-helix-inducing properties of this solvent (*18*).

We have previously shown that the 17 amino acid peptide (INRNAYGSMGGGSLRGS) at the hydrophilic C-terminal of the CsmA protein is exposed to the surface of the chlorosome (*11*). CD of this peptide showed a typical CD spectrum of a random coil with a negative peak at 200 nm, indicating that the C-terminal part of CsmA does not contribute to the measured α-helical content (results not shown).

Reconstitution of the CsmA−BChl a Complex. In order to reconstitute the baseplate CsmA−BChl *a* complex, CsmA protein was dissolved in detergent micelles of OG and a small volume of BChl *a* in acetone was added. The sample was incubated in the dark at 4 °C. We used BChl *a* extracted from *Rhodopseudomonas sphaeroides* R 26.1 since it has phytol as the esterifying alcohol like the BChl *a* in *Chl. tepidum* (*4*).

Formation of the reconstituted CsmA−BChl *a* complex was followed by a red-shift of the Q_y-band of BChl *a*, which shifted from 778 to 800 nm during 20 h incubation in the presence of CsmA (Figure 4, upper panel). No red-shift was observed in the control sample without CsmA (Figure 4, lower panel). During the 20 h incubation, a new peak at 689 nm appears in the spectra of both the preparations (Figure 4). BChl *a* is known to be extremely susceptible to chemical modification and photooxidation (*19*), so we presume that during the 20 h incubation, some degradation of the BChl *a* has occurred even when stored at 4 °C in the dark. It is noteworthy that in the control sample without CsmA (Figure 4, lower panel) formation of self-aggregated BChl *a* absorbing at 840 nm took place during the incubation.

Spectroscopic Properties of the Reconstituted Complex. The reconstituted CsmA−BChl *a* complex is extremely stable. In contrast to samples of BChl *a* in OG without CsmA, where BChl *a* degraded within 1 week, the spectral properties of the CsmA−BChl *a* complex were retained even

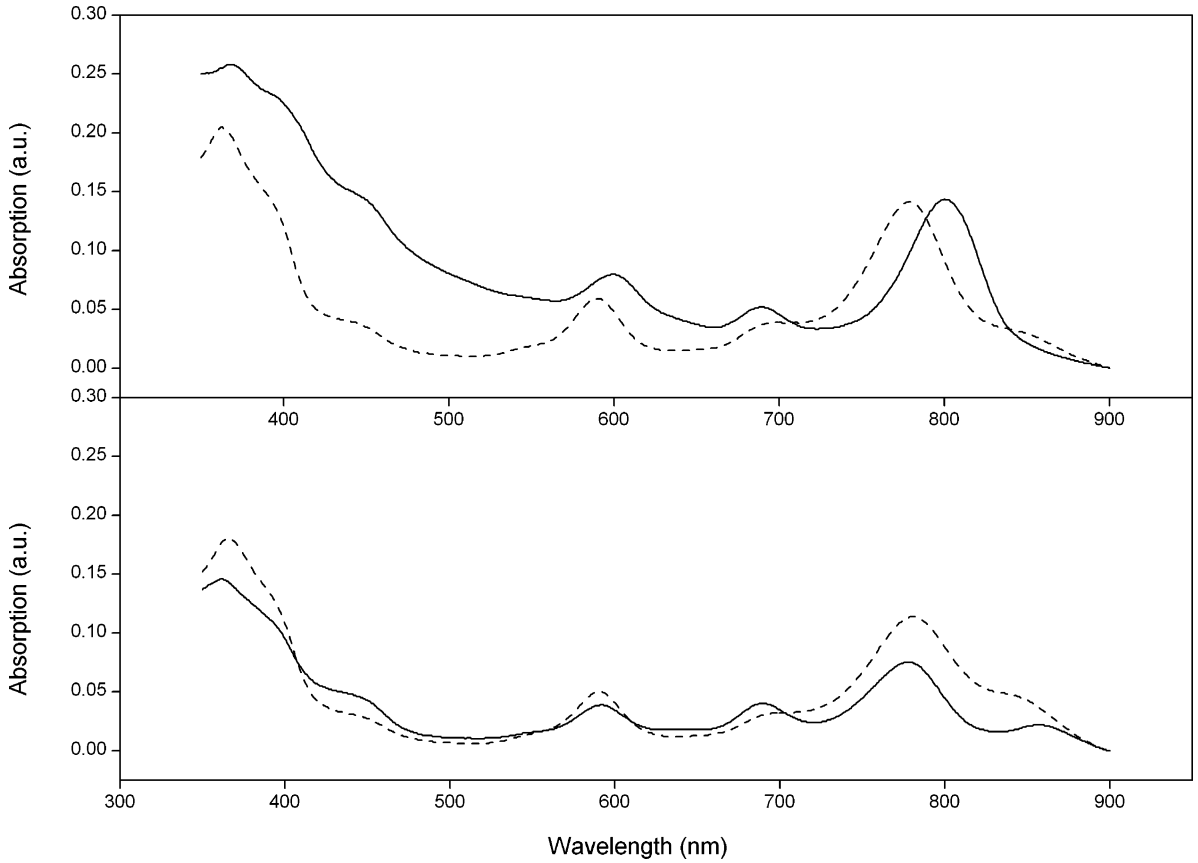

FIGURE 4: Reconstitution of baseplate CsmA−BChl *a* complex. In the top panel, absorption spectra of CsmA−BChl *a* reconstituted in 0.83% *n*-octyl *β*-D-glucopyranoside in 50 mM potassium phosphate buffer (pH 7.4) is seen after 0 h (dashed line) and 20 h (solid line). As a control, BChl *a* in 0.83% *n*-octyl *β*-D-glucopyranoside in 50 mM potassium phosphate buffer (pH 7.4) is seen in the lower panel, also after 0 h (dashed line) and 20 h (solid line). The CsmA concentration of the sample in the top panel was 10 μM, and the BChl *a* concentration was 5 μM in both experiments.

after storage for 4 months at 4 °C in the dark (results not shown).

We observed that the temperature had a strong effect on the position of the Q_y-band of the reconstituted CsmA−BChl *a* complex: It decreased linearly from 804 nm at 10 °C to 790 nm at 38 °C. In the same temperature range, the band position in the control sample of monomeric BChl *a* in OG decreased from 779 to 775 nm.

It was originally suggested that CsmA was a BChl *c*-binding protein (*20*). We therefore investigated whether CsmA is able to bind BChl *c* in the same way as BChl *a*. However, the spectral properties of monomeric BChl *c* did not change in the presence of the CsmA (results not shown).

To determine whether the conformation of the CsmA protein is changed upon binding to BChl *a*, we employed CD spectroscopy. Figure 5 shows a comparison of the CD spectra of the reconstituted CsmA−BChl *a* complex and the CsmA protein in OG micelles. Both spectra possess typical α-helix features with distinct negative bands at 208 and 222 nm. However, the shape and the intensity of the spectra are significantly different, indicating that BChl *a* induces a structural change in CsmA upon binding. On the basis of the ellipticity at 222 nm we estimated that binding of BChl *a* decreases the α-helix content of CsmA by one-third.

CD spectra were also recorded in the near IR region of bacteriochlorophyll absorption. Figure 6 shows the CD spectrum of the CsmA−BChl *a* complex and its corresponding absorption spectrum. The CD spectrum shows a positive

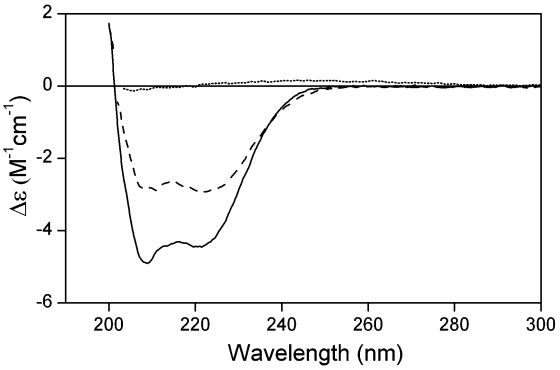

FIGURE 5: Secondary conformation of CsmA in the presence of BChl *a*. CD spectra of CsmA (solid line) and CsmA−BChl *a* complex (dashed line) were recorded after 24 h incubation at 4 °C in the dark. A CD spectrum of BChl *a* without protein is also shown (dotted line). CsmA and BChl *a* concentrations were 10 and 4 μM, respectively. All samples were prepared in 0.83% *n*-octyl *β*-D-glucopyranoside in 50 mM potassium phosphate buffer (pH 7.4).

signal at 795 nm and crosses the zero line at 805 nm. Additionally, a small negative feature at 820 nm could be observed that obviously could not be correlated to the absorption properties of the complex (Figure 6, top panel). Interestingly, the degradation product of BChl *a* absorbing at 689 nm present after the incubation for 20 h (see Figure 4) does not contribute to the CD spectrum of the complex. The near-IR spectrum of monomeric BChl *a* in OG was identical with the buffer baseline.

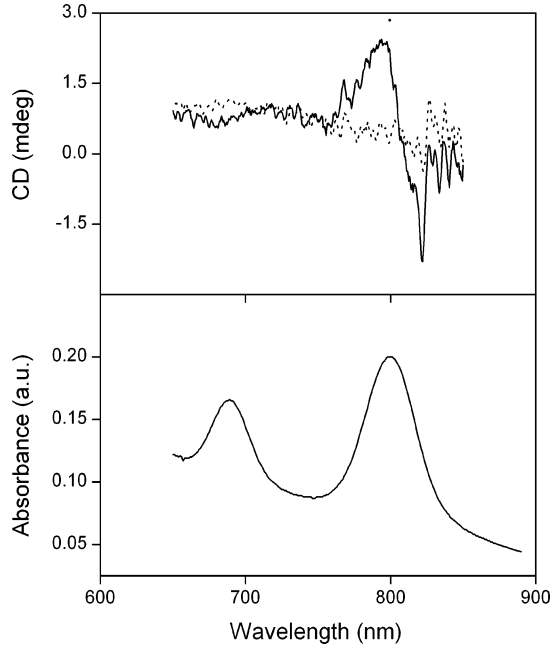

FIGURE 6: Near-IR CD spectroscopy of the CsmA−BChl *a* complex. The top panel shows NIR CD spectra of the CsmA−BChl *a* complex (solid line) and buffer (dotted line). The panel below shows the corresponding absorption spectrum to the CsmA−BChl *a* complex. Both CD and absorption spectra have been normalized at 900 nm. The CsmA and BChl *a* concentrations were 10 and 3 μM, respectively, and the sample was prepared in 0.83% *n*-octyl β-D-glucopyranoside in 50 mM potassium phosphate buffer (pH 7.4).

DISCUSSION

This is the first observation of a direct interaction between isolated CsmA protein and BChl *a*. Reconstitution of purified CsmA obtained from *Chl. tepidum* with BChl *a* in detergent micelles of OG clearly showed that the presence of CsmA causes a pronounced red-shift of the Q_y band of BChl *a* from 778 to ca. 800 nm, corresponding to the absorption properties of BChl *a* in the chlorosome baseplate. Previously, the Q_y absorption maxima for chlorosomal BChl *a* in various preparations of green sulfur bacteria have been reported: 795 nm in BChl *c*-depleted chlorosomes of *Chl. limicola* (*3*), 797 nm in carotenosomes of *Chl. tepidum* lacking BChl *c* (*8*), or 800 nm in whole cells of *Chlorobium vibrioforme* and *Prosthecochloris aestuarii* (*5*, *21*). In detergent-treated chlorosomes and the "minimal B798 baseplate" of *Cfx. aurantiacus*, the Q_y absorption maxima of BChl *a* is 795 and 798 nm, respectively (*6*, *7*).

The interaction between CsmA and BChl *a* in the OG detergent micelles was further confirmed by CD (Figure 6). The spectrum in the NIR shows a conservative signal with positive and negative peaks on either side of the absorption maximum at 800 nm. The calculated rotational strength $(A_L - A_R)/A$ of BChl *a* in the complex was $+3 \times 10^{-4}$ and -3×10^{-4}, respectively. It should be noted that BChl *a* is an intrinsically chiral molecule, as the result of the asymmetrically placed side groups of the porphyrin chromophore. However, in CD spectra of monomeric BChl *a* in either ether or in pyridine-containing carbon tetrachloride, the shape of the CD band resembles that of the corresponding absorption band with positive values for $(A_L - A_R)/A$ of 10^{-4} and 1.7×10^{-4}, respectively (*22*).

The CD spectrum shown in Figure 6 matches that reported for BChl *c*-deficient chlorosomes from *Chl. limicola (3)*, showing a conservative, symmetrical spectrum characteristic of excitonic interactions between at least two pigment molecules ($(A_L - A_R)/A$ of $+3.8 \times 10^{-4}$ and -3.8×10^{-4}, respectively); this suggestion was supported by fluorescence polarization measurements (*23*). In contrast, the CD spectrum of the "minimal B798 baseplate" of *Cfx. aurantiacus* (*7*) resembles that of the corresponding absorption band at 798 nm, but the CD band is negative instead of positive, as in the case of solutions of monomeric BChl *a* (*22*). We tentatively suggest that the CsmA−BChl *a* complex exists as a monomer in chlorosomes of the Chloroflexi and as a dimer in chlorosomes of the Chlorobi.

CD analysis of isolated CsmA in organic solvents (Figure 3) showed the presence of α-helix as the major structural motif, in agreement with the theoretical predictions made from the amino acid sequence (not shown). This secondary structure of CsmA was retained in micelles of OG, where the α-helical content of CsmA was approximately 40%. However, the α-helix content of CsmA decreased by approximately one-third after binding of BChl *a*. A possible explanation could be that the expected coordination of BChl *a* to His25 disturbs the α-helix of the neighboring residues. It is also possible that a slight rearrangement in the local environment around His25 induces altered aggregation properties. This would be in agreement with the CD data suggesting the presence of a CsmA−BChl *a* dimer.

We cannot exclude the possibility that some of the structural changes observed might arise from aggregation of the CsmA−BChl *a* complexes. Isolated CsmA is prone to form dimers on denaturating SDS polyacrylamide gels, and studies using zero-length cross-linkers have shown that CsmA in the intact chlorosome forms homo-oligomers up to at least octamers (*8*). It is noteworthy that after prolonged incubations (several days) we consistently observed a bluish-green deposit in the CsmA−BChl *a* preparations, indicative of formation of aggregates. This deposit could easily be resuspended and retained the same spectral properties as the original CsmA−BChl *a*. However, in absence of BChl *a*, no deposit of protein was observed (results not shown).

Equimolar ratios between CsmA and BChl *a* in both *Chl. tepidum* and *Cfx. aurantiacus* have been suggested previously (*24*, *7*). This is supported by earlier studies, where the stoichiometry between CsmA and BChl *a* was estimated to be 0.6−1.2 in *Chl. tepidum* and 1.4−2.7 in *Cfx. aurantiacus* (*6*). In spite of several attempts, we have not able to determine the exact stoichiometry between CsmA and BChl *a* in the reconstituted complex. Due to modifications of BChl *a* in the sample, the exact pigment concentration in the reconstituted complex could not be reliably determined during titration experiments. In addition, increasing the ratio between BChl *a* and CsmA consistently caused self-aggregation of BChl *a*, as shown in Figure 4.

Montaño et al. (*7*) showed that in the baseplate of *Chloroflexus aurantiacus* each CsmA−BChl *a* complex contains one or two carotenoid molecules. We therefore included a crude extract of carotenoids isolated from *Chl. tepidum* in our reconstitution experiments of the CsmA−BChl *a* complex. However, using steady-state fluorescence spectroscopy, we were not able to detect excitation energy transfer from the carotenoid to BChl *a* within the complex

(results not shown). Although carotenoids play an essential role in many photosynthetic systems, results have shown that a mutant of *Chl. tepidum*, which is not able to synthesize carotenoids, has a growth rate at low-light conditions that is reduced by only 25% of that of the wild-type (*25*). This suggests that in chlorosomes of green sulfur bacteria carotenoids may only play a structural role. In agreement with this, Ikonen et al. (*26*) recently concluded that carotenoids have a pronounced effect on the chlorosome structure, as observed by X-ray scattering and electron cryomicroscopy.

The procedures for reconstitution of the CsmA−BChl *a* complex were inspired by investigations of the LH2 light-harvesting antenna complex of purple bacteria (*27*−*29*). As is the case for CsmA, the α- and *β*-polypeptides of LH2 have molecular masses of around 6 kDa, show α-helix character (65%), and are amphipatic. In addition all three proteins have a conserved histidine (*29*). The Q_y absorption of the CsmA−BChl *a* complex around 800 nm is similar to B800 of LH2, which is situated in a quite polar environment (*30*, *31*). The major difference between the LH2 α- and *β*-polypeptides and CsmA is that the first have transmembrane-spanning α-helices, whereas CsmA is located in a lipid monolayer with both the C- and N-terminal exposed (*10*). We are currently using NMR to obtain structural information on the CsmA−BChl *a* complex. This complex is probably one of the simplest light-harvesting complexes and may be representative of the early stages in the evolution of photosynthesis.

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