Photosynth Res (2010) 104:233–243
DOI 10.1007/s11120-009-9519-y

REVIEW

A model of the protein–pigment baseplate complex in chlorosomes of photosynthetic green bacteria

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Received: 4 October 2009 / Accepted: 16 December 2009 / Published online: 14 January 2010

Abstract In contrast to photosynthetic reaction centers, which share the same structural architecture, more variety is found in the light-harvesting antenna systems of phototrophic organisms. The largest antenna system described, so far, is the chlorosome found in anoxygenic green bacteria, as well as in a recently discovered aerobic phototroph. Chlorosomes are the only antenna system, in which the major light-harvesting pigments are organized in self-assembled supramolecular aggregates rather than on protein scaffolds. This unique feature is believed to explain why some green bacteria are able to carry out photosynthesis at very low light intensities. Encasing the chlorosome pigments is a protein-lipid monolayer including an additional antenna complex: the baseplate, a two-dimensional paracrystalline structure containing the chlorosome protein CsmA and bacteriochlorophyll *a* (BChl *a*). In this article, we review current knowledge of the baseplate antenna complex, which physically and functionally connects the chlorosome pigments to the reaction centers via the Fenna–Matthews–Olson protein, with special emphasis on the well-studied green sulfur bacterium *Chlorobaculum tepidum* (previously *Chlorobium tepidum*). A possible role for the baseplate in the biogenesis of chlorosomes is discussed. In the final part, we present a structural model of the baseplate through combination of a recent NMR structure of CsmA and simulation of circular dichroism and optical spectra for the CsmA–BChl *a* complex.

Keywords Chlorosome · Baseplate · CsmA · Molecular modeling · Photosynthetic antenna

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Introduction

Green sulfur bacteria (GSB) and some filamentous anoxygenic phototrophs (FAP) share a unique photosynthetic antenna: the chlorosome (Blankenship and Matsuura 2003). Chlorosomes enable these organisms to grow by photosynthesis under extreme low light intensities, such as 100 m below the surface of the Black Sea (Manske et al. 2005), or at deep-sea hydrothermal vents (Beatty et al. 2005). A third type of chlorosome-containing bacteria in the phylum Acidobacteria was recently discovered: "*Candidatus* Chloracidobacterium thermophilum" (Bryant et al. 2007). Because these three groups of bacteria belong to distinct phylogenetic groups, it seems likely that the chlorosome genes have been transferred between the groups by horizontal gene transfer.

Chlorosomes are ellipsoid organelles with an interior of self-organized bacteriochlorophyll (BChl) aggregates. The

chlorosome is surrounded by an envelope, which contains an additional antenna complex, the CsmA–BChl *a* baseplate (Blankenship and Matsuura 2003), exclusively located on the surface toward the cytoplasmic membrane as a two-dimensional paracrystalline array. While the chlorosome architecture is similar in GSB and FAP, the link between the baseplate and the membrane bound reaction center (RC) differs. As seen in Fig. 1, GSB have a water-soluble antenna protein, the Fenna–Matthews–Olson (FMO) protein, linking the chlorosome baseplate to a type 1 RC, whereas in FAP the baseplate is connected to the B808–866 complex, an integral membrane component quite similar to light-harvesting complex 2 (LH2) of purple bacteria. This B808–866 complex interfaces with a type 2 RC. "*Candidatus* Chloracidobacterium thermophilum" has an FMO protein connected to a type 1 RC and produces chlorosomes under oxic conditions (Bryant et al. 2007) in contrast to GSB and FAP which carry out photosynthesis under anoxic conditions.

The chlorosome

The chlorosome contains aggregated bacteriochlorophylls and are the largest known photosynthetic antenna structure. Unlike other light-harvesting antennae like LH2 in purple bacteria or phycobilisomes in cyanobacteria, chlorosomes are heterogeneous structures and vary in size within the same cell. *Chlorobaculum* (*Cba.*) *tepidum* chlorosomes appear under transmission electron microscopy as organelles of 110–180 nm in length and 40–60 nm in width (Frigaard et al. 2004a; Li and Bryant 2009). It has been estimated that a typical chlorosome from *Cba. tepidum* contains approx. 200,000 BChl *c* molecules, 2,500 BChl *a* molecules, 20,000 carotenoid molecules, 18,000 quinone molecules, 5,000 protein molecules (of which half is CsmA), and 20,000 lipid molecules (Frigaard and Bryant 2006). Of these constituents, the chlorosome envelope is believed to contain all the proteins, BChl *a,* polar lipids, and part of the carotenoids and quinones. The low protein-to-pigment ratio when compared to other photosynthetic antenna systems means that the BChl *c* must occur as pigment aggregates rather than the usual organization on a protein scaffold.

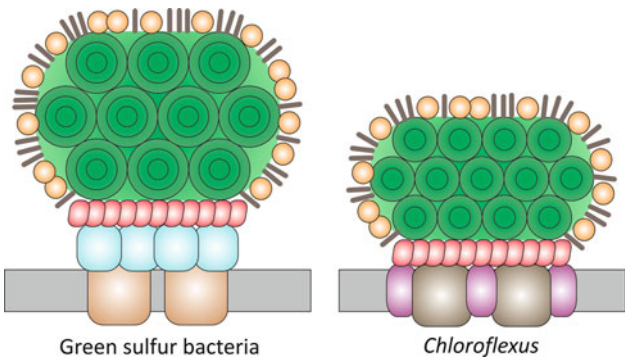

Fig. 1 Schematic representation of the photosynthetic apparatus of green sulfur bacteria (*left*) and filamentous anoxygenic phototrophs, represented by *Chloroflexus* (*right*). Self-organized bacteriochlorophyll *c*, *d*, or *e* (*green*) is surrounded by a protein-lipid monolayer (envelope proteins: *yellow*; lipids: *brown sticks*). The chlorosome baseplate (*red*) transfers excitation energy to either the Fenna–Matthews–Olson protein (*blue*) connected to a type 1 reaction center (*light brown*) or the B808–866 antenna (*purple*) connected to a type 2 reaction center (*dark brown*)

The organization of bacteriochlorophylls within the chlorosomes has been studied intensely for many years and arrangements as rod-like elements (Holzwarth and Schaffner 1994) or lamellar sheets (Psencik et al. 2004) have been proposed. Recently, a structure of the BChl *c* aggregates in *Cba. tepidum* was solved using cryo-EM, solid-state NMR, and ab initio modeling. This study showed that alternating BChl molecules form stacks assembled into layers with the farnesyl tails alternately extending on both sides and these stacks can either form coaxial cylinders or lamellar sheets (Ganapathy et al. 2009). How the aggregated BChls interact with other constituents of the chlorosomes remains uncertain.

The traditional picture of the chlorosome envelope as a lipid monolayer comes among others from the observed dimensions by electron microscopy, where the thickness appears to be only 3 nm (Staehelin et al. 1980). More than 50% of the total lipid content is made up of galactolipids (Sørensen et al. 2008) with the galactose moieties exposed on the chlorosome surface (Chung and Bryant 1996; Holo et al. 1985). Calculations done by Sørensen et al. (2008) reveal that the polar lipid content of *Cba. tepidum* chlorosomes is insufficient to cover the surface area of a chlorosome as a monolayer. It was therefore suggested that more than 90% of the chlorosome surface is covered with proteins with interdispersed lipids in a monolayer. Similar protein coating has been described for other bacterial inclusion bodies such as polyhydroxyalkanoate inclusions (Fuller 1999).

Progress in genetic manipulation of *Cba. tepidum* (Frigaard et al. 2004b) has led to a better understanding of the function of proteins and pigments in this light-harvesting organelle. Chlorosomes of *Cba. tepidum* contain 10 different proteins, all of which are located at the surface of the chlorosome envelope (for a recent review, see Frigaard and Bryant 2006). Mutants of *Cba. tepidum* have been constructed that lack a single gene (Frigaard et al. 2004b) or several genes (Li and Bryant 2009) encoding chlorosome proteins. Inactivation of *csmA* leads to non-viable cells in contrast to the genes for the other nine chlorosome proteins implying that CsmA, the major chlorosome protein, seems to be essential to the organism. All chlorosome protein mutants produced chlorosomes, but some mutants had varying contents of BChl *c* and varying chlorosome sizes. Accordingly, it was concluded that chlorosome

proteins other than CsmA play roles in determining the size of the chlorosome as well as the assembly and organization of the BChl *c* aggregates within the chlorosome (Li and Bryant 2009). It should be noted that there is no fixed stoichiometry between BChl *c* and any chlorosome protein. It has been proposed that the variety of types of chlorosome protein (other than CsmA) ensures that the protein-lipid envelope structure of the chlorosome is flexible by introducing irregularity and thereby preventing formation of a fully rigid organization like that of the paracrystalline baseplate (Frigaard and Bryant 2006).

CsmA is known to organize BChl *a* in the chlorosome baseplate and thus couple chlorosome BChls to the pigments in the RC. Knock-out studies clearly demonstrate that the baseplate is an essential part of the chlorosome envelope. In contrast, the cytoplasmic part of the envelope has a more variable nature and does not require an absolute stoichiometry of the nine chlorosome proteins for function. Even mutants lacking two of the envelope proteins are still able to assemble chlorosomes (Li and Bryant 2009). These findings are further emphasized in the *bchK* mutant of *Cba. tepidum* which is unable to synthesize BChl *c*. Chlorosomes of this mutant are reduced to so-called carotenosomes, which appear as small flat sacs only containing the CsmA–BChl *a* baseplate complex, carotenoids, and a minor amount of CsmD, but none of the additional eight chlorosome proteins and BChl *c* found in wild-type cells (Frigaard et al. 2005).

In conclusion, there are two physically distinct parts of the chlorosome envelope: the CsmA–BChl *a* baseplate appressed to the cell membrane, which is indispensable and can function independently as a light-harvesting antenna, and a part in contact with the cytoplasm which encases the BChl aggregates. The latter part is potentially variable in the sense that substantial changes in the structural proteins are possible without loss of function.

Biogenesis of the chlorosome

How chlorosomes are biosynthesized in cells is unknown and no observations of obvious intermediate structures in wild-type organisms are available. A hypothesis for chlorosome biogenesis was recently published by Hohmann-Marriott and Blankenship (2007). In this model, chlorosomes are considered as being specialized lipid bodies with a biogenesis similar to that of lipid bodies containing triacylglycerols, wax esters, and polyhydroxyalkanoates. The model predicts that BChls, along with carotenoids and quinones, accumulate between the two leaflets of the cytoplasmic membrane. Glycolipids accumulate within the membrane in the vicinity of the accumulated pigments. Further accumulation of glycolipids is paralleled by the association of chlorosome envelope proteins in the cytoplasmic membrane. Additional buildup of chlorosome constituents leads to the formation of a connected vesicle that finally separates from the cytoplasmic membrane as a mature chlorosome. This model appears to predict that if BChl *c* is not synthesized, the immature chlorosome may not separate from the cytoplasmic membrane and as a result chlorosome proteins and other chlorosome components would accumulate in the cytoplasmic membrane (or not accumulate in the cell at all) and no chlorosome-like structures would be formed in the cell.

However, this is contradicted by experimental observations in both GSB (Frigaard et al. 2005) and FAP (Foidl et al. 1998) of "empty" chlorosomes with no or very low contents of BChl *c*. Further contradictions come from the fact that BChl *c*, CsmA, and all other chlorosome proteins are not observed in the cytoplasmic membrane, but are exclusively located in the chlorosomes (Vassilieva et al. 2002). The lipid composition of chlorosomes is also markedly different from that of the cytoplasmic membrane (Schmidt 1980).

We propose an alternative model for chlorosome biogenesis, in which the properties of CsmA are essential (Fig. 2). We propose that as CsmA is synthesized it rapidly aggregates into a baseplate-like structure, which attaches to the FMO protein on one side, and accumulates chlorosome-specific lipids (predominantly glycolipids) on the other side forming an intermediate chlorosome (see below for details on CsmA oligomerization and interactions with the FMO protein). Attachments of the lipids create a hydrophobic pocket into which BChl *c* and chlorosome proteins other

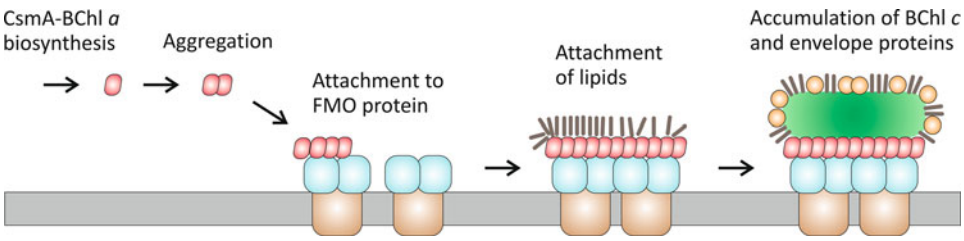

Fig. 2 A proposed pathway for chlorosome biogenesis in green sulfur bacteria. CsmA (*red*) self-aggregates immediately after biosynthesis, and subsequently anchors to the FMO protein (*blue*). Lipids (*brown sticks*) attach to the hydrophobic CsmA and create a hydrophobic interior in which BChl *c* (*green*) and additional chlorosome envelope proteins (*yellow*) accumulate

than CsmA accumulate in a concerted and interdependent manner. BChl *c* self-aggregates in the hydrophobic interior of the chlorosome and the chlorosome proteins accumulate in the envelope. The size of the chlorosome is determined by the extent of BChl *c* aggregation and by the organization of the chlorosome proteins in the envelope. If BChl *c* biosynthesis is decreased or disrupted, this model predicts that the chlorosome baseplate still forms, but that the chlorosome remains as an "empty bag".

The CsmA–BChl *a* baseplate complex

CsmA

CsmA is the most abundant chlorosome protein and accounts for around one-half of the total chlorosome protein content (Frigaard et al. 2004a). For many years, it was believed that the function of CsmA was to bind BChl *c* (Feick and Fuller 1984), and the CsmA protein is still incorrectly called "BChl *c*-binding protein" in many gene and protein databases. However, research within the past decade has shown that CsmA binds BChl *a*, and is located in the baseplate (Bryant et al. 2002; Montaño et al. 2003; Pedersen et al. 2008a; Sakuragi et al. 1999).

The 6.2 kDa CsmA protein is encoded by the *csmA* gene as a protein product of 79 amino acid residues. Post-translationally, CsmA is modified by removal of 20 amino acids from C-terminus (Chung et al. 1994; see Fig. 3). This process takes place after insertion into the chlorosome envelope, and pre-CsmA is present in the chlorosome envelope in small amounts as an 11th polypeptide (Vassilieva et al. 2002).

The atomic-resolution structure of CsmA has recently been determined by liquid-state NMR in a mixed solvent system (1:1 methanol:chloroform) and the average structure is seen in Fig. 3 (Pedersen et al. 2008b). The CsmA structure shows two α-helices: a long, amphipathic helix stretching over residues V6–L36 with a small bend around residue V29–M31, and a short helix stretching over residues M41–G49 in the C-terminal part of the protein. The conserved histidine (H25), believed to be involved in BChl *a* binding, is seen on the hydrophobic side of the helix. In this solvent system, CsmA displays no tertiary structure. The α-helix conformation has been confirmed by circular dichroism showing an α-helical structure in both organic solvents and in the presence of uncharged detergents (Pedersen et al. 2008a). Interestingly, recent solid-state NMR data from studies of ^{13}C, ^{15}N-labeled CsmA in carotenosomes has shown that no fundamental structural changes of the protein takes place when CsmA is situated in a native baseplate environment as compared to it being in solution in the mixed solvent system (Pedersen and Nielsen, manuscript in preparation).

As seen in Fig. 3, the CsmA protein is highly conserved both within the GSB and within the FAP. However, when comparing CsmA sequences between two groups, the overall homology is low, but there are two highly similar sequences: the GHW sequence including the histidine

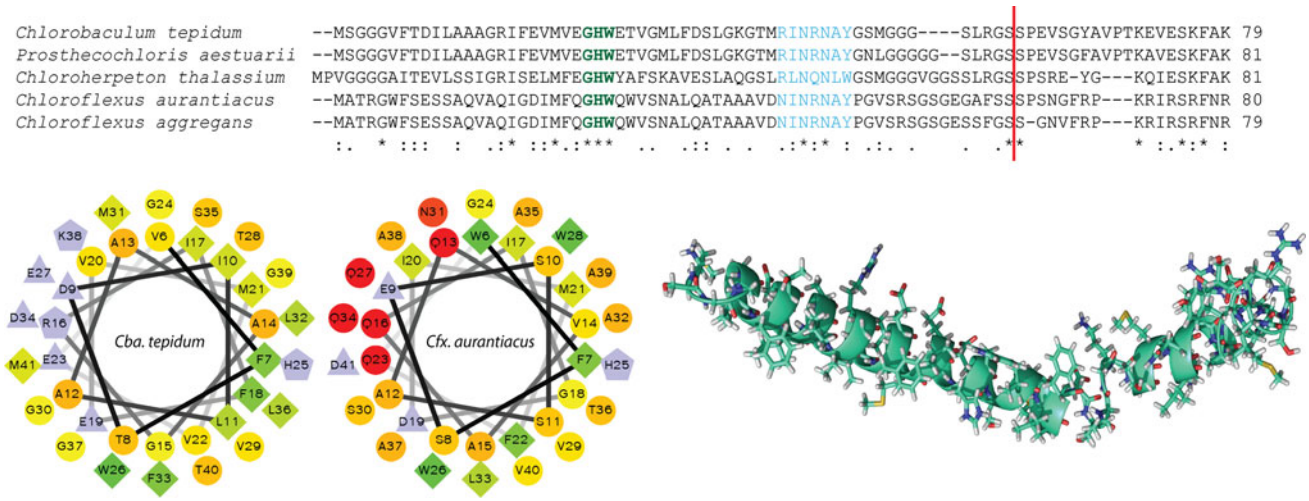

Fig. 3 CsmA from green sulfur bacteria (GSB) and filamentous anoxygenic phototrophs (FAP). *Top*: Sequence comparison of representatives of GSB (*Cba. tepidum*, *Prosthecochloris. aestuarii*, and *Chloroherpeton thalassium*) and FAP (*Cfx. aurantiacus*, *Cfx. aggregans*). The highly conserved sequence around the bacteriochlorophyll *a* binding His are shown in *green* and the conserved part of the C-terminal is shown in *blue*. The *red line* marks the site of post-translational cleavage. *Bottom*, *left*: Helical wheels of envelope embedded α-helices of CsmA from *Cba. tepidum* and *Cfx. aurantiacus* generated using the software wheel.pl (Armstrong and Zidovetzki 2001). The amino acids are shown as follows: charged (*light blue*; negative = *triangle*, positive = *pentagons*), hydrophilic (*circles*, *red* are most hydrophilic and *yellow* least hydrophilic) and hydrophobic (*diamonds*, *green* are most hydrophobic and *yellow* is least hydrophobic). *Bottom, right*: The liquid-state NMR structure of CsmA from *Cba. tepidum* (Pedersen et al. 2008b; pdb ID: 2K37)

believed to coordinate BChl *a*, and a region at the C-terminal, which in CsmA of *Cba. tepidum* adapts the short α-helix (M41–G49) seen in Fig. 3. The latter is believed to be involved in interactions with either the FMO protein (in GSB) or the B808–866 (in FAP) as discussed as follows. The structure of CsmA from *Cfx. aurantiacus* has not been determined, but sequence-based secondary structure analysis implies that CsmA from FAPs also would form amphipathic α-helices. Comparing α-helical wheels of CsmA from *Cba. tepidum* and *Cfx. aurantiacus* (Fig. 3), a noteworthy difference between CsmA of the two different groups of green bacteria is found in the hydrophilic amino acids of the amphipathic helix. In *Cba. tepidum*, a high content of charged amino acids is present on the hydrophilic side, whereas these have been replaced by polar glutamines in CsmA from *Cfx. aurantiacus*.

Topology of CsmA in the chlorosome envelope

CsmA is an atypical integral membrane protein due to its very amphipathic nature with a hydrophobic N-terminal region (residues 1–40) combined with a hydrophilic C-terminal region (residues 41–59; Chung et al. 1994; Milks et al. 2005). By digestion of *Cba. tepidum* chlorosomes with a mixture of various endo- and exopeptidases followed by mass spectrometry, it was found that the N-terminal hydrophobic residues (except for the initial methionine) were well-protected from proteolytic treatment and therefore must be buried deeply in the baseplate. In contrast, both the N-terminal M1 and residues M41–S59 from the C-terminal are easily cleaved and thus must be exposed on the surface (Milks et al. 2005). Interestingly, when similar experiments were carried out with chlorosomes of *Cfx. aurantiacus*, only residues 10–39 of CsmA were protected from proteases indicating a much more exposed N-terminus (L. Pham and M. Miller, unpublished data).

Interaction between CsmA and the FMO protein

The baseplate of *Cba. tepidum* is functionally connected to the FMO protein, which is bound to the RC in the plasma membrane. Since the baseplate is believed to consist of CsmA as the sole protein, it is reasonable to believe that the interaction between these two proteins ensures coupling of the chlorosomes to the RCs. This interaction has been confirmed by cross-linking studies, revealing that CsmA and the FMO protein are located in close proximity (Li et al. 2006).

As the C-terminal of CsmA protrudes out of the membrane, this is an obvious candidate for the CsmA–FMO interaction. A surface plasmon resonance study proved that a 17-residue synthetic peptide corresponding to the CsmA C-terminus does indeed interact specifically with the FMO protein (Pedersen et al. 2006). This interaction appears to be highly dependent on the conserved sequence found in the C-terminal (INRNAY for *Cba. tepidum*), and it was suggested that the interaction primarily takes place by means of hydrophilic interactions. Recently, it was shown that solvent water can penetrate in between the baseplate and the FMO protein, suggesting that FMO proteins are not very closely packed to the baseplate (Wen et al. 2009). This supports the suggestion of hydrophilic interactions making the most important contribution to the CsmA–FMO binding.

Crystal structures of the FMO protein from *Prosthecochloris (Ptc.) aestuarii* and *Cba. tepidum* reveal that FMO consists of three identical subunits enveloping seven BChl *a*, forming a trimer with a 3-fold symmetry axis (Camara-Artigas et al. 2003; Li et al. 1997; Matthews et al. 1979; Tronrud et al. 1986). The orientation of the FMO protein with respect to the baseplate and the membrane has been predicted by calculations showing that BChl *a* 3 and 4 must be situated closest to a reaction center in the membrane to ensure efficient energy transfer (Adolphs and Renger 2006). In this orientation, BChl *a* 1 is closest to the chlorosome, which was recently proved experimentally by mass spectrometry based foot printing (Wen et al. 2009).

It has been proposed that a trimer of FMO of *Cba. tepidum* binds an additional BChl *a* molecule (Ben-Shem et al. 2004). This was recently verified by Tronrud et al. (2009), who showed that an eighth BChl *a* is found in FMO proteins from both *Cba. tepidum* and *Ptc. aestuarii*. Separated from the FMO core pigments, the eighth BChl *a* is located in a cleft on the protein surface in a region close to the baseplate and with an orientation suggested to increase the energy-transfer efficiency between the baseplate and the FMO protein (Tronrud et al. 2009).

At present, there is no information on the interaction between CsmA and the light-harvesting B808–866 complex in the cytoplasmic membrane of *Cfx. aurantiacus.* However, the C-terminal sequence of CsmA, known to interact with FMO in *Cba. tepidum*, is also conserved *Cfx. aurantiacus*. It is reasonable to believe that the nature of the interactions is similar between the two groups.

Interactions between CsmA and BChl *a*

The first evidence for the presence of BChl *a* pigments in a baseplate connector between BChl *c* and the reaction center came from isolated chlorosomes of *Cfx. aurantiacus.* It was seen from absorption spectra that in chlorosomes of *Cfx. aurantiacus* the BChl *c* absorption band had a shoulder at 790 nm (Schmidt 1980), and shortly thereafter fluorescence spectroscopic measurements of intact *Cfx. aurantiacus* cells proved that this chlorosome-specific BChl *a*

actively facilitated energy migration from chlorosomes BChl *c* to BChl *a* of the reaction centers (Betti et al. 1982). In 1986, van Dorssen et al. (1986) showed that a similar pool of BChl *a* absorbing at 800 nm present in chlorosomes of *Chlorobium (Chl.) limicola* in addition to the much larger amount BChl *c*.

When different chlorosome fractions from *Chl. limicola* was analyzed by Gerola and Olson (1986), a major protein with an apparent MW of 4–5 kDa was always observed along with BChl *a,* despite depletion of BChl *c*. Therefore, they suggested that this protein was involved in the coordination of BChl *a*. Using selective extraction of chlorosomes, this protein was identified as being CsmA in both *Cba. tepidum* (Bryant et al. 2002) and *Cfx. aurantiacus* (Sakuragi et al. 1999). The baseplate from *Cfx. aurantiacus* was purified and found to consist of CsmA and BChl *a* along with carotenoids (Montaño et al. 2003). A reconstitution study between CsmA purified from *Cba. tepidum* and isolated BChl *a* reproduced the spectral properties of the native baseplate (Pedersen et al. 2008a). In chlorosomes of a *Cba. tepidum* mutant (*bchK*) incapable of synthesizing BChl *c*, it was noted that the CsmA–BChl *a* baseplate complexes are remarkably stable (Frigaard et al. 2005).

Histidines are known to coordinate the Mg^{2+} ion of chlorophylls in pigment proteins, as seen, for instance, in the FMO protein (Tronrud et al. 2009) and the light-harvesting antenna complexes of purple bacteria (Cogdell et al. 2004). Since all CsmA sequences from both GSB and FAP have a conserved histidine in the central part of the peptide (Fig. 3), it is believed that this site is responsible for pigment coordination. It was found that CsmA and BChl *a* exist in approximately equimolar amounts in the baseplate of different GSBs, consistent with a model where each His binds one BChl *a* (Bryant et al. 2002; Sakuragi et al. 1999; van Noort et al. 1994).

With these aspects in mind, we have used computer modeling to study the possible binding of BChl *a* to CsmA using our liquid-state NMR structure of pigment-free CsmA (Fig. 3). The calculations was initialized using molecular mechanical methods to test different Bchl *a* locations around the CsmA protein and find the minimum energy structure. The lowest energy structure was used as the basis for molecular dynamic simulations.

In the CsmA–BChl *a* model structure (Fig. 4), the Mg^{2+} atom of BChl *a* is coordinated to the H25 histidine and the C-3 acetyl group of BChl *a* forms a hydrogen bond with the W26 tryptophan residue. Hydrogen bonding between tryptophan and BChl *a* acetyl has been observed in LH1 complexes of *Rhodobacter sphaeroides*, and was found to cause the red-shift of protein bound BChl *a* versus free BChl *a* (Olsen et al. 1994; Sturgis et al. 1997). BChl *a* associated with CsmA is red-shifted by ~30 nm as

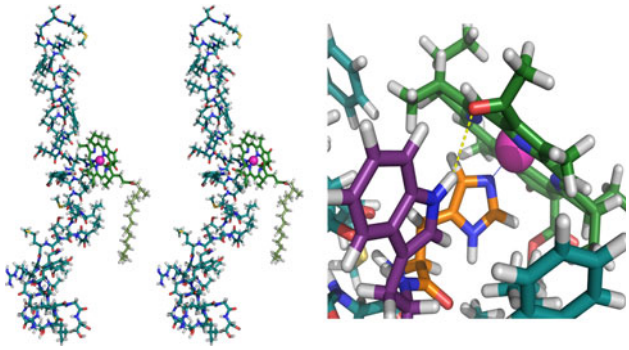

Fig. 4 Computer simulation of the interaction between CsmA and BChl *a* shown as a stereoview of the optimized structure (*left*) and in close-up (*right*). CsmA (*blue*) is shown with the the N-terminal region pointing upwards, and BChl *a* (*green*) is seen with the farnesyl chain pointing away from the protein. The central magnesium is highlighted in *magenta*. In the close-up, histidine 25 (*orange*) is seen coordinating the magnesium atom, and the hydrogen-bond from tryptophan 26 (*purple*) to the C-3 acetyl is visualized as *yellow dashes*. The CsmA-bacteriochlorophyll *a* model has been optimized using the molecular mechanics method with CHARMm27 force field and ab initio Hartree–Fock 6-311G** calculated atomic charges in the Tinker 4.2 software package (Ponder 2004). Visualized using PyMOL (DeLano 2002)

compared to BChl *a* in acetone, and it would be of interest to use genetic engineering to see whether the substitution of Trp by another aromatic amino acid would change the spectrum. In addition to W26, two tryptophans (W6 and W28) are found in the CsmA *Cfx. aurantiacus.* The presence of two tryptophans close to the conserved histidine might explain the ~5–10 nm blue shift of the BChl *a*-baseplate pigments of this organism as compared to *Cba. tepidum*.

Supramolecular structure of the CsmA and BChl *a* complex

It is believed that the CsmA–BChl *a* complex of the baseplate is arranged in a highly organized paracrystalline substructure, which has been seen as a striated pattern by freeze-fracture EM in both *Cba. tepidum* and *Chl. limicola*, as well as in chlorosomes from *Cfx. aurantiacus* (Oelze and Golecki 1995; Staehelin et al. 1980). The occurrence of a distinct baseplate lattice was clearly seen in chlorosomes of *Cfx. aurantiacus* using cryo-EM and X-ray diffraction (Pšenčík et al. 2009). As would be expected from a paracrystalline arrangement, CsmA is known to form oligomers in the chlorosome baseplate (Li et al. 2006) and the dimer formation is so strong that it has been observed even under the denaturating conditions used for SDS-PAGE (Frigaard et al. 2005; Vassilieva et al. 2002). Furthermore, BChl *a* in the baseplate also seems to interact in dimers, as seen from near infra-red circular dichroism (CD) spectroscopy of a baseplate preparation from *Chl. limicola* (Gerola and Olson

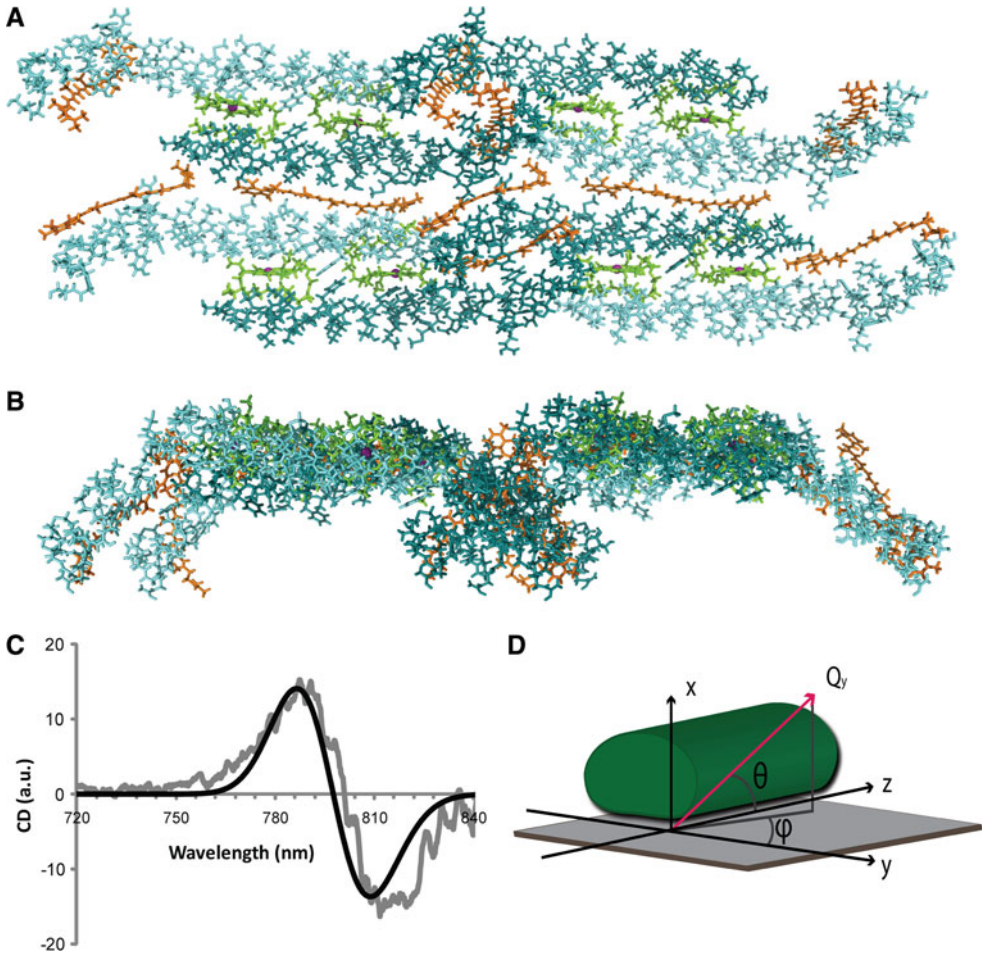

Fig. 5 Model of the chlorosome baseplate antenna. **a**, **b**: A simulated baseplate model of CsmA (alternating *dark* and *light blue*), BChl *a* (*green*), and carotenoid (*orange*) seen as a view onto the baseplate surface (**a**, *y*–*z* plane) and as a cross-section (**b**, *x*–*y* plane), in which the protruding C-terminal is seen sticking out of the surface. In the model, the stoichiometry between BChl *a* and carotenoids were 2:3. **c** Simulated CD spectrum (*black*) compared to an experimental CD spectrum of the CsmA–BChl *a* baseplate complex in a carotenosome preparation (*gray*). **d** Sketch of θ and φ angles describing orientation of the monomeric Q_y transition dipole vectors of BChl *a* with *z*-axis along the length of chlorosome (*green*). The plane of the baseplate is marked in *gray*. The model for the baseplate structure was calculated using classical MD simulations at 300 K and molecular mechanic method with CHARMm27 force field and ab initio Hartree–Fock 6-311G** calculated atomic charges in the Tinker 4.2 software package (Ponder 2004). In the exciton calculations, the absolute value of the monomeric Q_y transition dipole vector was taken as 6.13 [*D*], the dielectric constant was set to 2.1 [ε_0] and the monomeric Q_y transition energy (site energy) to 792 nm. Exciton stick spectra were dressed with a constant Gaussian shape function of width of 300 cm^{-1}. The size of the calculated baseplate model surface was 25 × 30 CsmA–BChl *a* dimer units. Visualization was performed in PyMOL (DeLano 2002)

1986) and a reconstituted complex between CsmA and BChl *a* in detergent micelles (Pedersen et al. 2008a). Furthermore, CD spectra of carotenosomes show excitonic interactions between BChl *a* molecules (Fig. 5c).

Linear dichroism of both *Cfx. aurantiacus* and *Chl. limicola* chlorosomes shows that the orientations of the transition moments of BChl *c* and *a* are basically different (Betti et al. 1982; van Dorssen et al. 1986), and it was assumed that the Q_y transitions of BChl *c* (742 nm) are oriented more or less parallel to the long axis of the chlorosomes, whereas the Q_y transitions of BChl *a* (798 nm) are perpendicular to the length axis. Recent fluorescence anisotropy measurements on single chlorosomes from *Cfx. aurantiacus* have shown the same arrangement, namely that monomeric Q_y transition dipole vectors of BChl *a* molecules are oriented almost perpendicular as compared to the main axis of the chlorosome (Shibata et al. 2007). The same phenomenon was observed in single chlorosomes of *Cba. tepidum*, which showed that slightly more declined from this main axis (Shibata et al. 2009).

Based on the CsmA structure, we previously suggested that dimerization might be driven by hydrophilic interactions between two helical cylinders (Pedersen et al. 2008b). These dimers would be arranged in long rows creating a paracrystalline array, and this would position two BChl *a* molecules in a hydrophobic pocket between two dimers

and thus allow for the observed pigment–pigment interaction. In this arrangement, the bacteriochlorin ring of BChl *a* is perpendicular to the baseplate surface, resulting in the closest connections to both BChl *c* aggregates in the chlorosome interior and BChl *a* 1 in the FMO protein, and fulfilling the observed constraints. Another possible arrangement would be to allow the hydrophilic part of the helix to face out of the chlorosome into an aqueous environment, but in this arrangement, BChl *a* appeared to be at an unfavorable angle to the BChl *c* molecules, and the distance to BChl *a* 1 would be too far in for efficient energy transfer.

Model of the chlorosome baseplate

In order to model the chlorosome baseplate, we have used the CsmA–BChl *a* unit from Fig. 4 to generate different structures with slightly different orientations and intermolecular distances between BChl *a* molecules. The model was based on a close interaction between two mirrored CsmA–BChl *a* units with a BChl *a* dimer sandwiched between two CsmA proteins, which were positioned parallel to the surface of the baseplate, as suggested from experimental data (Milks et al. 2005; Pedersen et al. 2008b). These complexes are aligned to produce a large sheet of CsmA-BChl *a* units. In order to evaluate this model, we simulated the spectroscopic properties of BChl *a* molecules in the model using structural parameters obtained from molecular mechanical optimizations and exciton theory with transition dipole approximation as previously described (Linnanto et al. 1999). One of the best-fitting baseplate models is presented in Fig. 5. As shown in Fig. 5c, the simulated CD spectrum correctly reproduces an experimental CD spectrum of BChl *a* in the baseplate of a carotenosome preparation. Since carotenoids are believed to be present in the baseplate (Arellano et al. 2000; Melø et al. 2000), these have been included in our model as discussed below.

In our model, the orientation of BChl *a* in the baseplate is described by the angles θ and φ of the vertically oriented monomeric Q_y transition dipole vectors. θ is the angle between the monomeric Q_y transition dipole vector and the main axis of the baseplate, and φ is angle between the projection of the monomeric Q_y transition vector on the baseplate surface and the vector on the baseplate surface perpendicular to the main axis of the baseplate (Fig. 5d). Calculations revealed that the shape of the simulated CD spectrum reproduced the experimental spectra when choosing intervals of θ angels between $60° \leq \theta \leq 90°$ or $90° \leq \theta \leq 120°$, for the two mirror image BChl *a* molecules in a CsmA–BChl *a* dimer unit, and φ angles of $0° \leq \varphi \leq 40°$ or $0° \leq \varphi \leq -40°$, for the two θ angle intervals, respectively.

However, the values for both θ and φ affected the calculated magnitude of the rotational strength. Also, the intermolecular distances of the BChl *a* dimers in baseplate models had an influence on magnitude of the rotational strength. It increases as distance decreases from about 2.7–1.5 nm which are the distances obtained from the present molecular modeling analysis. For the model in Fig 5, the intradimer distance is 1.8 nm, $\theta = 80°$ (110°), $\varphi = 10°$ (−10°), and the rotational strength $(A_L - A_R)/A$ can be calculated as about $\pm\ 3 \times 10^{-4}$ which is in good agreement with a previously reported experimental value for the reconstituted CsmA–BChl *a* complexes (Pedersen et al. 2008a).

Our calculated values for the orientation of the BChl *a* molecules within the baseplate model can be compared with results obtained from fluorescence anisotropy measurements on single chlorosomes from *Cba. tepidum* (Shibata et al. 2009). This study revealed that the transition dipole vectors of aggregated BChl *c* molecules in rod elements along the baseplate axis and BChl *a* in the baseplate were at an angle of 77° (θ). This value is in excellent agreement with the value of θ (60°–90°) obtained in our simulations.

A close interaction between CsmA and carotenoids in the baseplate has been reported from studies of carotenoid-depleted chlorosomes of *Chl. phaeobacteroides* and *Cba. tepidum* (Arellano et al. 2000; Melø et al. 2000). Also, based on structural analysis of chlorosomes derived from a carotenoid-deficient mutant of *Cba. tepidum*, it was suggested that carotenoids function as a "molecular glue" in the assembly of CsmA protein into the baseplate (Ikonen et al. 2007). Interestingly, when we purify CsmA on a gel filtration column, the protein always elutes in a yellow fraction (Pedersen et al. 2008a), which implies that interaction between the CsmA protein and carotenoids is very strong.

In our computer simulations of the baseplate structure, we therefore included chlorobactene, the most abundant carotenoid in chlorosomes of *Cba. tepidum* (Frigaard and Bryant 2006). We varied the chlorobactene/BChl *a* ratio between zero and four. All resulting simulations had a common structural characteristic with a basic structure based on CsmA–BChl *a* dimers. In simulations where chlorobactene was included, it was energetically favorable to position one chlorobactene molecule into the groove between adjacent CsmA proteins of separate dimers as shown in Fig. 5. In simulations with chlorobactene/BChl *a* ratios greater than 0.5, excess carotenoid molecules located around to C-terminal regions of CsmA proteins as shown in Fig. 5.

It is interesting to note that this baseplate structure is very likely to be conserved among different GSB, as they all share very similar CsmA proteins. For FAP, the

baseplate is likely to be organized in a quite different manner: first, CsmA from *Cfx. aurantiacus* has been suggested to form only dimers as observed by cross-linking (Feick and Fuller 1984) and not oligomers as observed in GSB. Second, it is estimated that chlorosomes from *Cfx. aurantiacus* contain about 2 BChl *a* molecules per CsmA protein as compared to the equimolar ratio in *Cba. tepidum* (Montaño et al. 2003; Sakuragi et al. 1999) implying that the conserved histidine is not the only BChl *a* binding group. Third, the CD spectrum of the isolated baseplate shows no derivative shape signal characteristic of exciton coupling, indicating the BChl *a* molecules do not form an interacting dimer (Montaño et al. 2003). These differences might be the direct result of the structural differences between the CsmA protein in *Cfx. aurantiacus,* which has a less charged α-helix (Fig. 3) and a more exposed N-terminal as compared to *Cba. tepidum* CsmA.

Concluding remarks

The baseplate, which forms the functional link between the self-aggregated bacteriochlorophylls within the chlorosomes and the FMO protein, is very simple compared to other light-harvesting antennae, with a structural element formed of only a single, small polypeptide, the 5.9 kDa CsmA protein, one BChl a molecule, and one or two carotenoids. We have presented a model for the *Cba. tepidum* chlorosome baseplate, which is consistent with experimentally determined optical parameters of the BChl *a* and structural information on the CsmA protein. In the model, CsmA coordinates BChl *a* through a conserved histidine (H25) with a conserved tryptophan (W26) forming a hydrogen bond to the C-3 acetyl of the porphyrin ring. Units of BChl *a*–CsmA form dimers with two closely spaced BChl *a* molecules sandwiched between two CsmA proteins. These dimers then form higher oligomers resulting in formation of a 2D lattice, in which the alpha helix of CsmA is aligned parallel to the surface. We suggest that the oligomerization may be stabilized by carotenoids.

The BChl *a* in a *Cba. tepidum* cell distributes with ~60% in the FMO protein, ~30% in the CsmA protein, and ~10% in the reaction centers (Frigaard et al. 2003). Therefore, it could be anticipated that cells may survive in the absence of CsmA if the only function it light harvesting. However, attempts to genetically eliminate CsmA from *Cba. tepidum* have repeatedly failed (Chung et al. 1998; Frigaard et al. 2004a; Li and Bryant 2009). It is possible that the chlorosome baseplate fulfills an essential functional, and possibly structural, role in the photosynthetic apparatus in green sulfur bacteria, even in the absence of BChl *c* aggregates in the chlorosome.

Based on the presence of a homodimeric type 1 reaction center complex, GSB are thought to be among the first photosynthetic cells (Ben-Shem et al. 2004). This implies that chlorosomes also might be representatives of one of the earliest antenna systems. Understanding the structural arrangement of CsmA–BChl *a* in the baseplate might therefore provide fundamental knowledge of the general evolution of basic antenna systems and serve as inspiration for construction of artificially light-harvesting devices.

Acknowledgments We thank Dr R. P. Cox for critical reading of the manuscript. This study was supported by the Danish National Research Foundation by grants to NCN, NUF, and MM. Support from Academy of Finland (contract No. 123801) is acknowledged by JL.

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