CsmA Protein is Associated with BChl *a* in the Baseplate Subantenna of Chlorosomes of the Green Photosynthetic Bacterium *Oscillochloris Trichoides* Belonging to the Family *Oscillochloridaceae*

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Abstract: The idea of association of bacteriochlorophyll *a* (BChl *a*) with protein in chlorosomes of the photosynthetic green anoxygenic filamentous bacterium *Osc.trichoides*, member of the family *Oscillochloridaceae*, was probed by low-temperature fluorescence spectroscopy and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis of alkaline-treated and untreated chlorosomes. Alkaline treatment of *Osc.trichoides* chlorosomes resulted in disappearance of BChl *a* band in their fluorescence spectra. The determination of BChl *c* and BChl *a* content confirmed the removal of BChl *a* from *Osc.trichoides* chlorosomes upon alkaline treatment. Based on the data obtained, we concluded that alkaline treatment of chlorosomes destroys the BChl *a* in the baseplate while leaving BChl *c* in a form that is spectrally indistinguishable from that in untreated chlorosomes. It was shown that upon alkaline treatment, only the 5.7 kDa CsmA protein was removed from the chlorosomes among five proteins detected by SDS-PAGE analysis, concomitantly with the disappearance of BChl *a* fluorescence emission. Based on these results, we suggest that CsmA protein is associated with BChl *a* in the baseplate subantenna in the chlorosomes of the photosynthetic green bacterium *Osc. trichoides*. Comparison of the data for the three families of green photosynthetic bacteria is relevant to assessing the universal principles of optimal antenna organization preserved in evolution despite marked changes in antenna size and structure.

Keywords: *Oscillochloridaceae*; Chlorosome; Baseplate; Bacteriochlorophylls *a* and *c*; CsmA protein

Introduction

The photosynthetic apparatus of green anoxygenic bacteria has a particular molecular organization and contains chlorosomes, unique extra membrane light-harvesting antennae structures that are attached to the inner surface of the cell membrane (Bryant and Frigaard, 2006). Chlorosomes are ellipsoid bodies (70–260 nm long, 30–100 nm wide and 10–30 nm thick) that are surrounded by a monolayer lipid–protein envelope. The bulk of light-harvesting pigments, bacteriochlorophylls (BChl) *c*/*d e* and carotenoids, is located within chlorosomes. These BChl *c/d/e* oligomers form either rod-(with a diameter of 5–10 nm) (Staehelin *et al.*, 1978, 1980) or lamellar-like structures (Pšenčik *et al*., 2004, 2006, 2009), arranged parallel to the longer chlorosome axis. A minor amount of BChl *a* pigments, absorbing at 795–800 nm, is also present in the chlorosome. This BChl *a* is located in the baseplate, observed in freeze-fracture electron-micrographs of chlorosomes from *Chloroflexaceae* and *Chlorobiaceae* species as a 5–6 nm thick paracrystalline layer (Staehelin *et al*., 1978, 1980). This BChl *a* serve as an intermediate antenna component, transferring excitation energy from chlorosomal BChl *c/d/e* to membrane-bound BChl *a* (Blankenship, Matsuura *et al*., 2003).

The baseplate is believed to be a pigment-protein

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complex that is located at the base of the chlorosome (Bryant and Frigaard, 2006). Chlorosomes from the green filamentous bacterium *Cf . aurantiacus* have been reported to contain three major proteins, CsmA, CsmM and CsmN, with molecular masses 5.7, 11 and 18 kDa (Sakuragi *et al*., 1999). CsmA is the smallest and most abundant of these proteins. In green sulfur bacteria (*Chl. tepidum*, *Chl. vibrioforme*, and *Chl. phaeobacteroides*) ten chlorosome proteins have been identified. The 6.2-kDa CsmA accounts for about half of the protein present in the chlorosome (Bryant and Frigaard, 2006).

In this work, the idea of association of BChl *a* with protein in chlorosomes of *Osc. trichoides* was probed by low-temperature fluorescence spectroscopy and SDS-PAGE analysis of alkaline-treated and untreated chlorosomes. We showed that the baseplate BChl *a* subantenna does exist in *Oscillochloridaceae* chlorosomes as a complex of BChl *a* with the 5.7 kDa CsmA protein.

Materials and Methods

Osc. trichoides DG-6, the type strain of the species *Osc. trichoides* (327 KM MGU), was grown as described earlier, in batch cultures with stirring under anaerobic conditions at 30 °C on a modified DGN medium at a moderate light intensity (50 μE m^{-2} s^{-1}) from incandescent lamps (Taisova *et al*., 2002).

Chlorosomes were isolated from *Osc. trichoides* cells through two successive continuous sucrose gradient (55%–20% and 45%–15%) in the presence of 10 mmol sodium ascorbate and 2 mol sodium thiocyanate as described earlier (Taisova *et al*., 2002).

Absorption spectra were recorded at room temperature with a Hitachi-557 spectrophotometer (Japan). Fluorescence emission spectra were measured at liquid nitrogen temperature (77 K) with a Hitachi-850 spectrometer. Excitation wavelength was 720 nm. The absorbance of the samples of chlorosomes was 0.2 at 750 nm. Before fluorescence measurements the chlorosomes were incubated 60 min with 20 mmol sodium dithionite at 4 °C to ensure strongly reduced conditions (up to −400 mV).

Quantitative BChl *a* and BChl *c* contents were determined according to the method developed by (Feick *et al.*, 1982).

Chlorosomes were treated with alkali according to the method developed by Van Walree *et al.* (1999).

Proteins from alkaline-treated and untreated chlorosomes were separated by SDS-PAGE analysis. Chlorosome samples were extracted with 1.4 ml of acetone at −20 °C overnight. Proteins were collected by centrifugation and dissolved in sample buffer [50 mmol Tris-HCl (pH 8.6), 24% (v/v) glycerol, 8% (w/v) SDS, 2% (v/v) 2-mercaptoethanol, and 0.1% (w/v) bromophenol blue]. The samples were boiled for 1 min before being loaded onto gels containing 16.5, 10 and 4% acrylamide as separating, spacer and stacking gel, respectively, as described by Schägger and van Jagow 1987. After electrophoresis, the gels were stained with Coomassie brilliant blue R-250 (CBB).

Results and Discussion

To degrade selectively the baseplate BChl *a* in *Osc. trichoides* chlorosomes we applied the method of alkaline treatment (Van Walree *et al*., 1999). Fig. 1 shows the effect of alkaline treatment (dotted line) on the absorption spectrum of the *Osc. trichoides* chlorosomes. Obviously, that the absorption bands of BChl *c*, the main light-harvesting pigment in *Osc. trichoides* chlorosomes, were not affected by alkaline treatment.

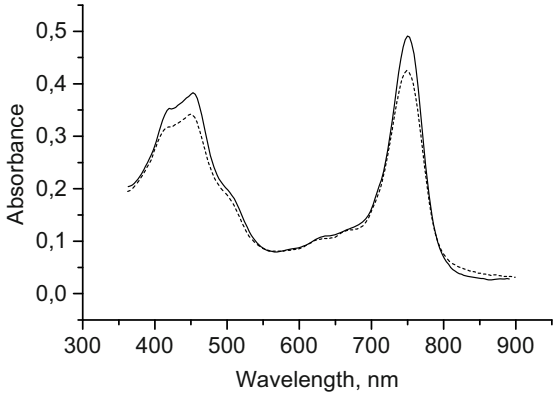

Fig. 1 Absorption spectra of untreated (solid line) and alkaline-treated (dotted line) *Osc. trichoides* chlorosomes in 50 mmol tris-buffer (pH 8.0).

In contrast to absorbance spectra, BChl *a* emission could be discerned in the fluorescence emission spectra of *Osc. trichoides* chlorosomes at 77 K (but not at room temperature) (Taisova *et al*., 2002). Additionally, it was shown by us that the light-harvesting *Osc. trichoides* chlorosome antenna exhibited a highly redox-dependent BChl *c* fluorescence similar to *Chlorobiaceae* species

(Taisova *et al*., 2002). For this reason, fluorescence emission spectra of untreated and alkaline-treated chlorosomes were measured at 77 K under reducing conditions (dithionite, 20 mmol) after excitation in the Q_y-band of BChl *c* at 720 nm.

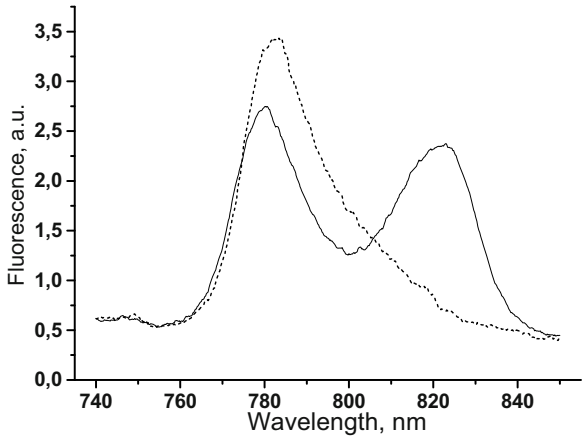

Fig. 2 Fluorescence emission spectra of untreated (solid line) and alkaline-treated (dotted line) *Osc. trichoides* chlorosomes at 77 K under reducing conditions.

Fig. 2 shows that alkaline treatment of *Osc. trichoides* chlorosomes resulted in disappearance of BChl *a* band in the 821 nm spectral region in their fluorescence spectra measured at 77 K (dotted line). The determination of BChl *c* and BChl *a* content confirmed the removal of BChl *a* from *Osc. trichoides* chlorosomes upon alkaline treatment (data not shawn). We conclude that alkaline treatment of chlorosomes destroys the BChl *a* in the baseplate while leaving BChl *c* in a form that is spectrally indistinguishable from that in untreated chlorosomes.

The effects of alkaline treatment on *Osc. trichoides* chlorosomal proteins were analyzed by SDS-PAGE analysis. Study of protein composition of *Osc. trichoides* chlorosomes were carried out in comparison with *Cfx .aurantiacus* chlorosomes. Five proteins (three major and two minor) were detected in native *Osc. trichoides* chlorosomes (Fig. 3A, lane 2). It was shown that upon alkaline treatment, only the 5.7 kDa CsmA protein was removed from the chlorosomes among five proteins detected by SDS-PAGE analysis (Fig. 3, lane 3), concomitantly with the disappearance of BChl *a* fluorescence (Fig. 2, dotted line), leaving BChl *c* unchanged spectrally. The protein composition of native *Cfx. aurantiacus* chlorosomes and its changes after alkaline treatment are shown in Fig. 3B. It is seen, that protein profiles of untreated and alkaline-treated *Osc. trichoides* and *Cfx. aurantiacus* chlorosomes were very much alike**.** In view of this, we designated the proteins of *Osc.trichoides* chlorosomes similarly to the proteins of *Cfx.aurantiacus* chlorosomes: CsmA (5.7 kDa), CsmM (11 kDa) and CsmN (18 kDa). Selective BChl *a* and 5,7 kDa protein disappearance should be expected only in case when both of them are located out of the BChl *c* body. Based on the results obtained, we suggest that CsmA is associated with BChl *a* in the baseplate subantenna in the chlorosomes of the green mesophilic filamentous photosynthetic bacterium *Osc. trichoides*, member of the new family *Oscillochloridaceae*.

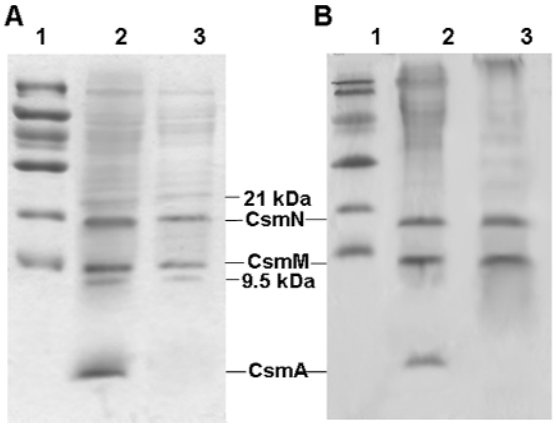

Fig. 3 CBB-stained SDS-PAGE of untreated and alkaline-treated *Osc. trichoides* (A) and *Cfx. aurantiacus* chlorosomes (B). A: Lane 1, molecular markers. Lane 2, untreated *Osc. trichoides* chlorosomes; Lane 3, alkaline-treated *Osc. trichoides* chlorosomes; B: Lane 1, molecular markers. Lane 2, untreated *Cfx. aurantiacus* chlorosomes; Lane 3, alkaline-treated *Cfx. aurantiacus* chlorosomes. All *Cfx. aurantiacus* samples were adjusted to contain 4.5 μg BChl *c*, while *Osc. trichoides* samples were adjusted to contain 18 μg BChl *c*.

The presented results support the idea that the baseplate subantenna, representing a complex of BChl *a* with a ~6 kDa CsmA protein, is a universal interface between the BChl *c* subantenna of chlorosomes and the light-harvesting BChl *a* subantenna of the cytoplasmic membrane in all three known families of green anoxygenic photosynthetic bacteria (*Chloroflexaceae*, *Oscillochloridaceae* and *Chlorobiaceae*). The group of chlorosome-containing bacteria with this type of baseplate organization was enlarged by the recently discovered new phototrophic chlorosome-containing organism *Candidatus* Chloracidobacterium thermophilum from the phylum *Acidobacteria* (Bryant *et al*., 2007).

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References

Blankenship RE, Matsuura K (2003) Antenna Complexes from Green Photosynthetic Bacteria. In: Green BR, Parson WW (eds.), Light-Harvesting Antennas in Photosynthesis. Kluwer Academic Publishers: Dordrecht, pp. 195-217

Bryant DA, Costas AMG, Maresca JA, Chew AGM, Klatt CG, Bateson MM, Tallon LJ, Hostetler J, Nelson WC, Heidelberg JF, Ward DM (2007) Candidatus Chloracidobacterium Thermophilum: an Aerobic Phototrophic Acidobacterium. Science 31**7:** 523-526

Frigaard NU, Bryant DA (2006) Chlorosomes: Antenna Organelles in Photosynthetic Green Bacteria. In: Shively JM (ed.), Complex Intracellular Structures in Prokaryotes. Microbiology Monographs, Vol. 2. Springer: Berlin, Germany, pp. 79-114

Feick RG, Fitzpatrick M, Fuller RC (1982) Isolation and Characterization of Cytoplasmic Membranes and Chlorosomes from the Green Bacterium Chloroflexus Aurantiacus. J. Bacteriol. 150(2): 905-915

Oelze J, Golecki JR (1995) Membranes and Chlorosomes of Green Bacteria: Structure, Composition and Development. In: Blankenship RE, Madigan MT, Bauer CE (eds.), Anoxygenic photosynthetic bacteria. Kluwer Academic Publishers: Dordrecht, pp. 259-278

Pšenčik J, Arellano JB, Ikonen TP, Borrego CM, Laurinmäki P, Butcher SJ, Serimaa RE, Tuma R (2006) Internal Structure of Chlorosomes from Brown-Colored Chlorobium Species and the Role of Carotenoids in Their Assembly. Biophys. J. 91: 1433-1440

Pšenčik J, Ikonen TP, Laurinmäki P, Merckel MC, Butcher SJ, Serimaa RE, Tuma R (2004) Lamellar Organization of Pigments in Chlorosomes, the Light Harvesting System of Green Bacteria. Biophys. J. 87: 1165-1172

Psencík J, Collins AM, Liljeroos L, Torkkeli M, Laurinmäki P, Ansink HM, Ikonen TP, Serimaa RE, Blankenship RE, Tuma R, Butcher SJ (2009) Structure of chlorosomes from the Green Filamentous Bact Crium Chloroflexus Aurantiacus. J. Bacteriol. 191: 6701-6708

Sakuragi Y, Frigaard NU, Shimada K, Matsuura K (1999) Association of Bacteriochlorophyll a with the CsmA Protein in Chlorosomes of the Photosynthetic Green Filamentous Bacterium Chloroflexus Aurantiacus. Biochim. Biophys. Acta 1413: 172-180

Schägger H, van Jagow G (1987) Tricine-Sodium Dodecyl Sulfatepolyacrylamide Gel Electrophoresis for the Separation of Proteins in the Range from 1 to 100 kDa. Anal. Biochem. 166: 368-379

Staehelin LA, Golecki JR, Fuller RC, Drews G (1978) Visualization of the Supramolecular Architecture of Chlorosomes (Chlorobium Type Vesicles) in Freeze-Fractured Cells of Chloroflexus Aurantiacus. Arch. Microbiol. 119: 269-277

Staehelin LA, Golecki JR, Drews G (1980) Supramolecular Organization of Chlorosomes (Chlorobium Vesicles) and of Their Membrane Attachment Sites in Chlorobium Limicola. Biochim. Biophys. Acta 589: 30-45

Taisova AS, Keppen OI, Lukashev EP, Arutyunyan AM, Fetisova ZG (2002) Study of the Chlorosomal Antenna of the Green Mesophilic Filamentous Bacterium Oscillochloris Trichoides. Photosynth. Res. 74: 73-85

Van Walree CA, Sakuragi Y, Steensgaard DB, Bosinger CS, Frigaard NU, Cox RP, Holzwarth AR, Miller M (1999) Effect of Alkaline Treatment on Bacteriochlorophyll a, Quinones and Energy Transfer in Chlorosomes from Chlorobium Tepidum and Chlorobium Phaeobacteroides Photochem. Photobiol. 69: 322-328