The original publication is available at www.springerlink.com
DOI 10.1007/s002030100258

Archives of Microbiology

DOI 10.1007/s002030100258

Original Paper

Effect of carotenoid deficiency on cells and chlorosomes of *Chlorobium phaeobacteroides*

Juan B. Arellano · Carles M. Borrego · Asunción Martínez-Planells · Librado Jesús Garcia-Gil(✉)

J.B. Arellano · C.M. Borrego · A. Martínez-Planells · L.J. Garcia-Gil
Laboratory of Microbiology, Institute of Aquatic Ecology and Department of Biology, University of Girona, 17071, Girona, Spain

✉ E-mail: garciagil@morgat.udg.es
Phone: +34-972-418175
Fax: +34-972-418150

Received: 21 September 2000 / **Revised:** 23 January 2001 / **Accepted:** 25 January 2001 / **Published online:** ▮

Abstract. The effects of inhibition of carotenoid biosynthesis by 2-hydroxybiphenyl on the photosynthetic growth, pigment composition and chlorosome structure of *Chlorobium phaeobacteroides* strain CL1401 were examined. At a concentration of 20 µg 2-hydroxybiphenyl $\cdot ml^{-1}$, carotenoid synthesis was largely inhibited (85%), but the photosynthetic growth rate was almost unaffected ($\mu_{control}$ =0.00525±0.00007 h^{-1} and $\mu_{HBP\text{-}treated}$ =0.00505±0.0005 h^{-1}). Cells grown in the presence of the inhibitor were 5 µm-70 µm long, while control cells were between 2-5 µm long. Moreover, 2-hydroxybiphenyl-treated cells contained fewer, unevenly distributed chlorosomes per µm of cytoplasmic membrane with an irregular arrangement (2.5±1.5 vs of 9.1±1.9). This was concomitant to the 83% decrease in the content of bacteriochlorophyll (BChl) *e* in 2-hydroxybiphenyl-treated cells. Electron microscopy revealed that the shape of carotenoid-depleted chlorosomes changed from ellipsoidal to spherical, although the mean volume was similar to that of control chlorosomes. SDS-PAGE analysis of the chlorosome polypeptide composition showed that the amount of CsmA protein decreased by 60% in carotenoid-depleted chlorosomes. This was paralleled by a decrease in the baseplate BChl *a* content. The data suggest that carotenoids are close to the chlorosomal baseplate, where they carry out both structural and photoprotective functions.

Keywords. Baseplate - Bacteriochlorophyll, Carotenoid - Chlorosome - CsmA - Green sulfur bacteria - Isorenieratene

Abbreviations. *BChl*: Bacteriochlorophyll *Cfl.*: *Chloroflexus Chl.*: *Chlorobium FMO*: Fenna-Matthews-Olson *HBP*: 2-Hydroxybiphenyl

Introduction

Green anoxygenic photosynthetic bacteria comprise two distinct phylogenetic groups, the green sulfur bacteria (*Chlorobiaceae*) and the green filamentous bacteria (*Chloroflexaceae*), which share a unique light-harvesting complex: the chlorosome. These vesicles are oblong bodies attached to the inner side of the cytoplasmic membrane (Blankenship et al. *1995*; Olson *1998*). Chlorosomes vary considerably in size depending on the bacterial species, growth conditions, and developmental stage of the cell (Oelze and Golecki *1995*). Green sulfur bacterial chlorosomes range from 70 to 180 nm in length and from 30 to 60 nm in width, whereas the chlorosomes from green filamentous bacteria are about 100 nm and 20-40 nm wide (Oelze and Golecki *1995*). Chlorosomes contain a large amount of bacteriochlorophyll (BChl) *c*, *d*, or *e*, which are made up of a series of homologous molecules differing in the degree of alkylation of the chlorine ring at positions C-8, C-12 and C-20, and the esterifying alcohol at C-17 (Senge and Smith *1995*; Blankenship et al. *1995*). The chemical structure of these homologues allows the spontaneous formation of cylindrical aggregates, the so-called rod elements, without the need for proteins or other chromophores (Holzwarth et al. *1990*; Griebenow et al. *1991*; Holzwarth and Schaffner *1994*). The chlorosome envelope consists of a glycolipid monolayer, mostly monogalactosyldiglycerol (Holo et al. *1985*), and a few small proteins, the Csm family (Wullink et al. *1991*; Chung and Bryant *1996*).

Together with the main chlorosomal BChls, BChl *a* and carotenoids are also present in chlorosomes in different amounts. BChl *a* forms a baseplate on the cytoplasmic membrane side and is thought to be associated with the CsmA protein (Sakuragi et al. *1999*). This BChl acts as an intermediate pigment in the energy transfer from the chlorosome to the cytoplasmic-membrane-embedded reaction center (Blankenship et al. *1995*). In green sulfur bacteria, an additional layer consisting of a water-soluble BChl *a* protein, the Fenna-Matthews-Olson (FMO) protein, connects the chlorosomal baseplate to the reaction center (Olson *1998*). Aryl carotenoids such as chlorobactene and OH-chlorobactene or isorenieratene and β -isorenieratene are the main carotenoids in chlorosomes of green- and

brown-colored sulfur bacteria, respectively, while γ - and β -carotene prevail in *Chloroflexus*

(Liaaen-Jensen *1965*; Schmidt *1980*; Overmann et al. *1992*). The amount of these pigments within the antenna is highly variable and depends on the bacterial species, light conditions, and stage of development (Schmidt *1980*; Oelze and Golecki *1995*; Frigaard et al. *1997*; Takaichi et al. *1997*; Frese et al. *1997*; Borrego et al. *1999*).

Carotenoids are known to play two essential roles in green photosynthetic bacteria. (1) Singlet energy transfer from carotenoids to BChls has been reported to be high in *Chloroflexus* (*Cfl.*) *aurantiacus*, *Chlorobium* (*Chl.*) *phaeovibrioides* and *Chl. tepidum* (Van Dorsen et al. *1986*; Otte et al. *1991*; Melø et al. *2000*), but low in *Chl. phaeobacteroides* (Cox et al. *1998*). (2) It has been suggested that carotenoids partly quench the BChl triplet state in chlorosomes (Psencík et al. *1994*; *1997*; Carbonera et al. *1998*). Recently, it has been reported that BChl *a*, rather than BChl *e*, receives photoprotection from carotenoids in chlorosomes (Arellano et al. *2000b*). However, little is known about the precise location of carotenoids within the chlorosomes. Foidl et al. (*1997*), and Frese et al. (*1997*) suggested that, in chlorosomes of *Cfl. aurantiacus*, the bulk of the carotenoids were in the vicinity of BChls and might contribute to the attachment of chlorosomes to the cytoplasmic membrane.

In the present study, the effect ofthe inhibition of carotenoid biosynthesis on the photosynthetic growth, pigment composition and chlorosome structure of *Chl. phaeobacteroides* strain CL1401 was investigated. Special attention was given to BChl *a*, as this pigment decreases in the absence of carotenoids in chlorosomes. The results support the hypothesis that carotenoids are located in the vicinity of the baseplate, where they carry out both structural and photoprotective functions.

Material and methods

Organism and growth conditions

Chlorobium phaeobacteroides strain CL1401 was grown in Pfennig mineral medium (Trüper and Pfennig *1992*) under continuous illumination (average light intensity of 100 µmol photons $m^{-2} \cdot s^{-1}$) provided by two Philips SL25 fluorescent lamps at the surface of the culture bottle. Inhibition of carotenoid biosynthesis was carried out with 2-hydroxybiphenyl according to Foidl et al. (*1997*). Growth of the control and 2-hydroxybiphenyl-treated cultures was followed by measuring the protein concentration (Lowry et al. *1951*) as a function of time. Cells were harvested by centrifugation at 23,500 × *g* for 15 min at 4 °C in a Sorvall RC-5B at the beginning of the stationary phase, when

cultures had a protein content of about 100 µg protein·ml^{-1} and had been growing for at least three generations. Cell pellets were washed in 50 mM Tris-HCl, pH 8.0, and 2 M NaSCN and stored at -80 °C. The absorption spectra of cells were recorded in a Fujitsu UV-2501PC UV-Vis spectrophotometer. Cell morphometry was observed by phase-contrast microscopy (Axioskop, Zeiss, Jena, Germany).

Isolation of chlorosomes

Chlorosomes of *Chl. phaeobacteroides* were isolated on a sucrose gradient prepared in 50 mM Tris-HCl, pH 8.0, and 2 M NaSCN (Gerola and Olson *1986*). A further purification step was carried out using a flotation sucrose gradient (Steensgaard et al. *1997*). Chlorosomes were pooled and stored at -80 °C.

Pigment analysis

Photosynthetic pigments were analyzed by reverse-phase HPLC according to Borrego and Garcia-Gil (*1994*) with minor modifications described in Arellano et al. (*2000a*). BChl *e*, BChl *a*, and carotenoid concentrations were calculated from the area of their corresponding peaks monitored at 473 nm, 770 nm, and 453 nm, respectively. The HPLC system was calibrated by injecting pure pigments dissolved in ethanol, the concentrations of which were first determined in a Fujitsu UV-2501PC UV-Vis spectrophotometer. The molar extinction coefficients in ethanol were 62,800 for BChl *a* at 774 nm (Connolly et al. *1982*), 41,000 for BChl *e* at 654 nm, and 107,000 for Isr at 450 nm (Borrego et al. *1999*).

Analysis of chlorosome polypeptide composition

Polyacrylamide gel electrophoresis was carried out using a Protean II xi Cell (Bio-Rad Laboratories) according to Laemmli (*1970*) with a total acrylamide content of 17.5% in the separating gel. Chlorosomes were precipitated with 10% trichloroacetic acid and the pigments were washed out with cold acetone. Samples were suspended in sample buffer and incubated for 5 min at 100 °C. The gels were stained with Coomassie R-250, and the intensity of protein bands was measured using a Gel Doc

1000 Gel documentation system (Bio-Rad Laboratories).

Electron microscopy

The length and width of chlorosomes was measured on ultra-thin frozen sections of cells. Cells were collected at the stationary phase by filtration through a 0.22-µm pore diameter nylon filters (MSI Science) and processed according to Tokuyasu (*1978*). Samples were embedded in 10% gelatin and then fixed in 2.5% glutaraldehyde. After cryoprotection with 2.3 M sucrose, the samples were stored overnight at 4 °C. Ultra-thin sections were obtained using a Reichert-Jung Leica cryo-ultramicrotome and frozen by plunging them into liquid nitrogen. Ultra-thin frozen sections were collected and placed on carbon-coated grids, placed on ice, and covered by a 2% gelatin membrane. Sections were stained and stabilized in a 3% aqueous uranyl acetate/2% methylcellulose solution and observed in a Hitachi 600AB transmission electron microscope. The images were recorded digitally with a Bioscan 792 camera (Gatan). The number of chlorosomes in cells was referred to µm of cytoplasmic membrane for comparison purposes.

Likewise, purified chlorosomes were observed in a Zeiss 910 transmission electron microscope. Chlorosomes were first dialyzed against 50 mM Tris-HCl, pH 8.0, to remove sucrose. Negative staining was done with 1% uranyl acetate. Chlorosome length and width were measured on micrographs of negatively stained chlorosomes using SCAN-PRO image analysis software (Jandel Scientific). Chlorosome height was measured on cell-section micrographs. The volume of the chlorosomes was assumed to be the same as that of an ellipsoid ($V=^{4}/_{3}\,\pi\,abc$, where *a*=half length, *b*=half width, *c*=half height).

Results

Effect of 2-hydroxybiphenyl on growth

The concentration of 2-hydroxybiphenyl that led to maximal inhibition of carotenoid biosynthesis without significantly affecting growth was determined. At 20 µg 2-hydroxybiphenyl ml^{-1}, inhibition of the specific carotenoid content was about 98%. The specific content of carotenoids perceptibly decreased, but so did the specific content of BChl *e* (83%) and BChl *a* (96%) (Table 1). Although 2-hydroxybiphenyl specifically inhibits carotenoid biosynthesis, it has also been shown to hinder BChl *c* and BChl *a* formation (Foidl et al. *1997*). However, the 2-hydroxybiphenyl concentration used did not inhibit the photosynthetic growth rate, as shown in Fig. 1. Concentrations above 20 µg 2-hydroxybiphenyl $\cdot ml^{-1}$ clearly inhibited growth of *Chl. phaeobacteroides* (data not shown).

Table 1. Molar pigment ratios and specific pigment content (in nmols pigment (mg protein)$^{-1}$) of control and HBP-treated cells of *Chlorobium phaeobacteroides*

Cells	BChl e	Car	BChl a	BChl e:Car	BChl e:BChl a
Control	115.1±5.8	52.5±3.5	5.2±0.7	2.2±0.3	22.8±3.9
Treated	19.7±0.2	1.3±0.06	0.2±0.01	15.5±0.6	81.2±2.7

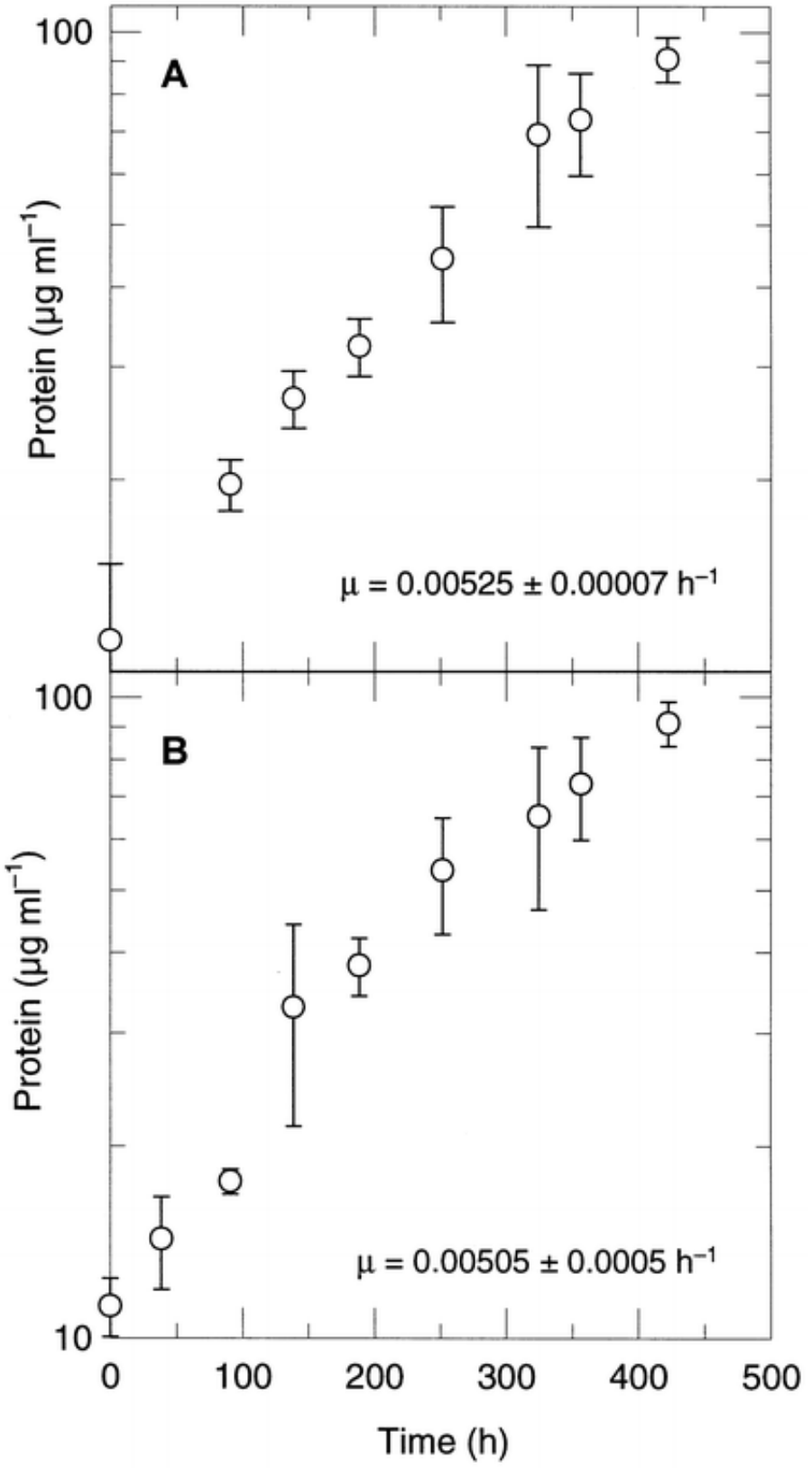

Fig. 1. Growth kinetics of (**A**) control and (**B**) 2-hydroxybiphenyl (20 µg ml^{-1}) treated cultures of *Chlorobium phaeobacteroides* CL1401. Each data point represents the mean protein concentration of duplicated cultures; *bars* standard deviation of the mean

Treatment with 2-hydroxybiphenyl had noticeable effects on cell morphology, with-treated cells exhibiting a wide variety of morphologies ranging from rods (0.8 × 4 µm) to long filaments (0.8 × 70 µm). By contrast, control cells showed the standard rod morphology of the species studied (0.8 × 3-4 µm) (Fig. 2). These changes in the morphology were possibly caused by an effect of 2-hydroxybiphenyl on cell division, but this was not verified experimentally.

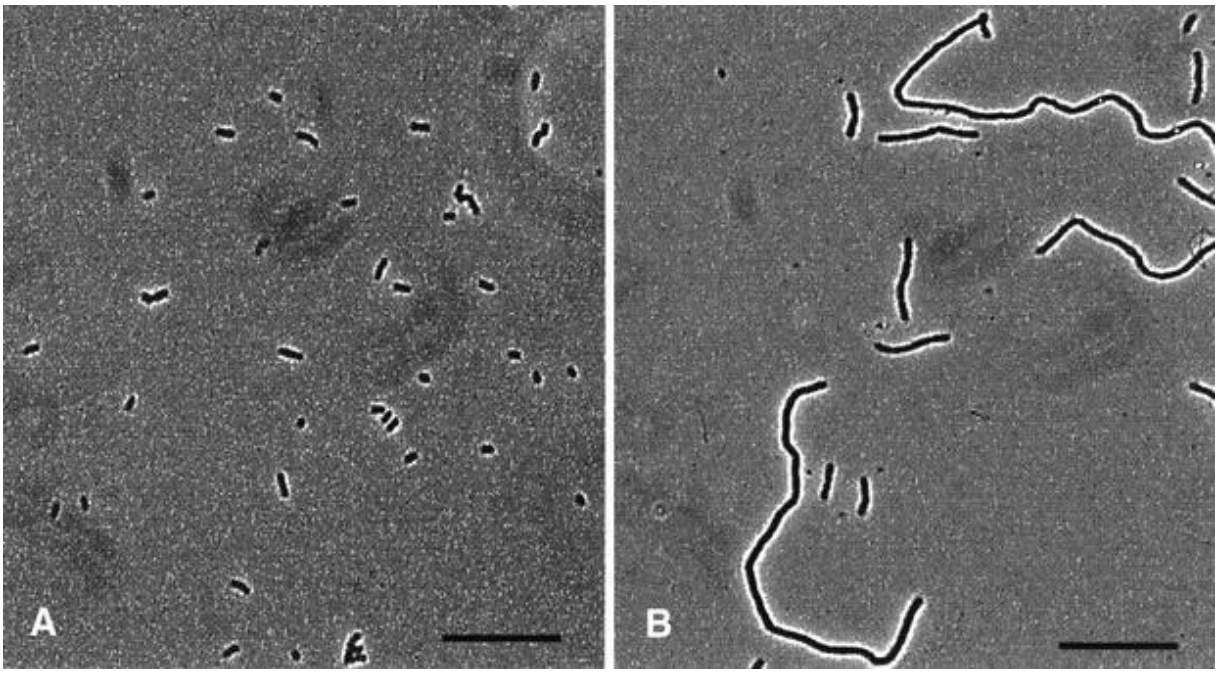

Fig. 2. Micrographs of control (**A**) and carotenoid-deficient (**B**) cells of *Chl. phaeobacteroides* CL1401. *Bar* 10 µm

Pigment analysis

A comparison of the absorption spectra of control and 2-hydroxybiphenyl-treated cells revealed changes in the spectral properties of the latter. These spectral changes were very similar to those reported for carotenoid-depleted chlorosomes (Arellano et al. *2000a*). First, the BChl *e* Q_y peak in 2-hydroxybiphenyl-treated cells was blue-shifted 6 nm, which was attributed to a change in the angle between the transition dipole of the BChl *e* Q_y and the long axis of the rod (Arellano et al. *2000a*). Second, the intensity of the BChl *a* Q_y band around 800 nm was lower. And third, the absorption band in the blue region of 2-hydroxybiphenyl-treated cells indicated a lack of carotenoids. However, the overlap between the absorption spectrum of isorenieratene and the split Soret band of BChl *e* in aggregate arrays (Arellano et al. *2000b*) hindered the assessment of carotenoid inhibition by simply comparing the two absorption spectra. Instead, this was thoroughly examined by HPLC analysis of control and 2-hydroxybiphenyl-treated cells. The latter displayed the typical elution pattern for BChl *e* (Borrego and Garcia-Gil *1994*; Arellano et al. *2000a*), but differed notably from control cells in the carotenoid region. 2-Hydroxybiphenyl-treated cells also had little isorenieratene and β -isorenieratene but accumulated the colorless precursor phytoene (data not shown). As deducedfrom the molecular ratios between BChl e and carotenoids and between BChl e and BChl a, carotenoids and BChl *a*, respectively, decreased by 86% and 72% in the photosynthetic units of 2-hydroxybiphenyl-treated cells (Table 1).

Polypeptide composition

To determine whether 2-hydroxybiphenyl treatment altered the polypeptide composition of chlorosomes, protein profiles of isolated chlorosomes from *Chl. phaeobacteroides* were analyzed by SDS-PAGE (Fig. 3). The resulting polyacrylamide gel revealed a number of small polypeptide bands, ranging from about 35 kDa to 6 kDa, which were assigned to the Csm protein family. However, traces of other polypeptides with large molecular masses were also present even when two consecutive sucrose gradients were carried out to purify chlorosomes. Polypeptides in the 40-60 kDa region were attributed to the FMO and the reaction center proteins as judged by their molecular mass. CsmA is a polypeptide observed in all *Chlorobiaceae* and *Chloroflexaceae* species and is usually the smallest chlorosomal protein band in an SDS-PAGE analysis. Here it was identified by comparing the chlorosomal polypeptide patterns of *Chl. tepidum* and *Chl. phaeobacteroides* (data not shown). In Fig. 3, the CsmA protein displayed a broad band with an apparent molecular mass of 4.5±0.5 kDa, which was smaller than the 6.3 kDa molecular mass determined by protein sequencing (Wagner-Huber et al. *1988*). Other proteins of the Csm family, i.e. CsmB and CsmE, were not traceable in chlorosomes from *Chl. phaeobacteroides*, suggesting that these two polypeptides occur, if at all, in smaller amounts than in chlorosomes of *Chl. tepidum*. In addition, two protein bands of around 10 and 35 kDa were observed, as also described by Van Walree et al. (*1999*) and by Stolz et al. (*1990*). Comparison of the polypeptide pattern of control (Fig. 3, lane A) and carotenoid-depleted (Fig. 3, lane B) chlorosomes revealed that the latter had a 60% decrease in the intensity of the CsmA protein but a slight increase in the 20 kDa protein.

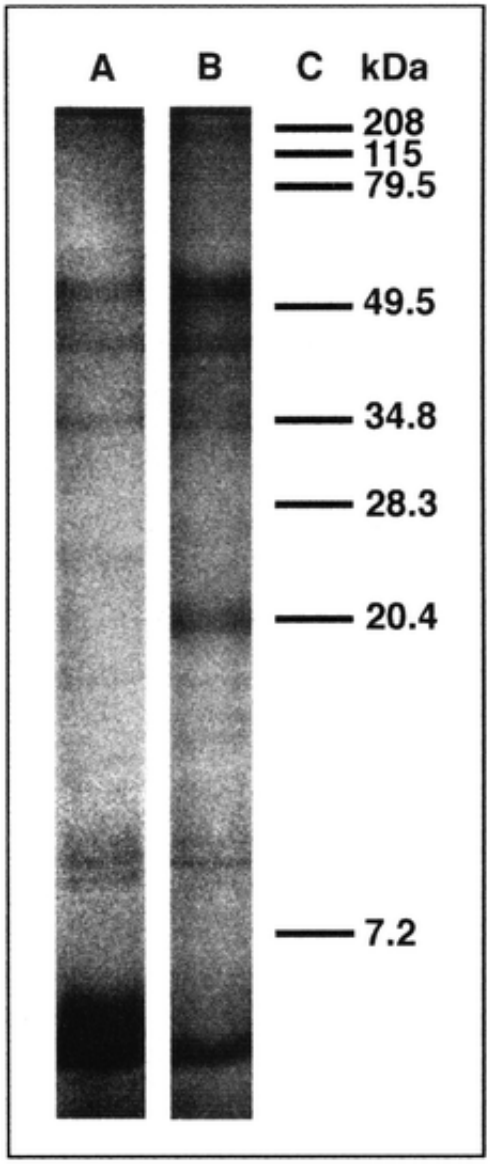

Fig. 3. SDS-PAGE analysis of the polypeptide composition of chlorosomes of *Chl. phaeobacteroides* strain CL1401. Proteins were applied in amounts corresponding to 50 µg of BChl *e* per lane and visualized by Coomassie blue R-250. *Lanes*: *A* Control chlorosomes, *B* carotenoid-depleted chlorosomes, *C* broad-range molecular mass standard (Bio-Rad Laboratories)

Chlorosome morphology and biometrics

Transmission electron microscopy (TEM) micrographs of control and carotenoid-depleted chlorosomes are shown in Fig. 4A and 4B, respectively. Control chlorosomes had the standard ellipsoidal shape and dimensions of these vesicles (Oelze and Golecki *1995*; Olson *1998*). However, the carotenoid-depleted chlorosomes had a shorter length, a similar width, and a larger height (Table 2). As a result, carotenoid-depleted chlorosomes became more rounded but they maintained a mean volume similar to that of control chlorosomes (Table 2). TEM micrographs of control cells showed a continuous array of chlorosomes attached to the inner side of the cytoplasmic membrane (Fig. 4C). This arrangement clearly differed from that of the 2-hydroxybiphenyl-treated cells, which

exhibited a more irregular distribution of chlorosomes along the cytoplasmic membrane (Fig. 4D). The number of chlorosomes per µm of cytoplasmic membrane was 9.1±1.9 and 2.5±1.5 for control and 2-hydroxybiphenyl-treated cells, respectively. Apart from the uneven arrangement of chlorosomes along the cytoplasmic membrane in 2-hydroxybiphenyl-treated cells, few chlorosomes were detached from the membrane (data not shown).

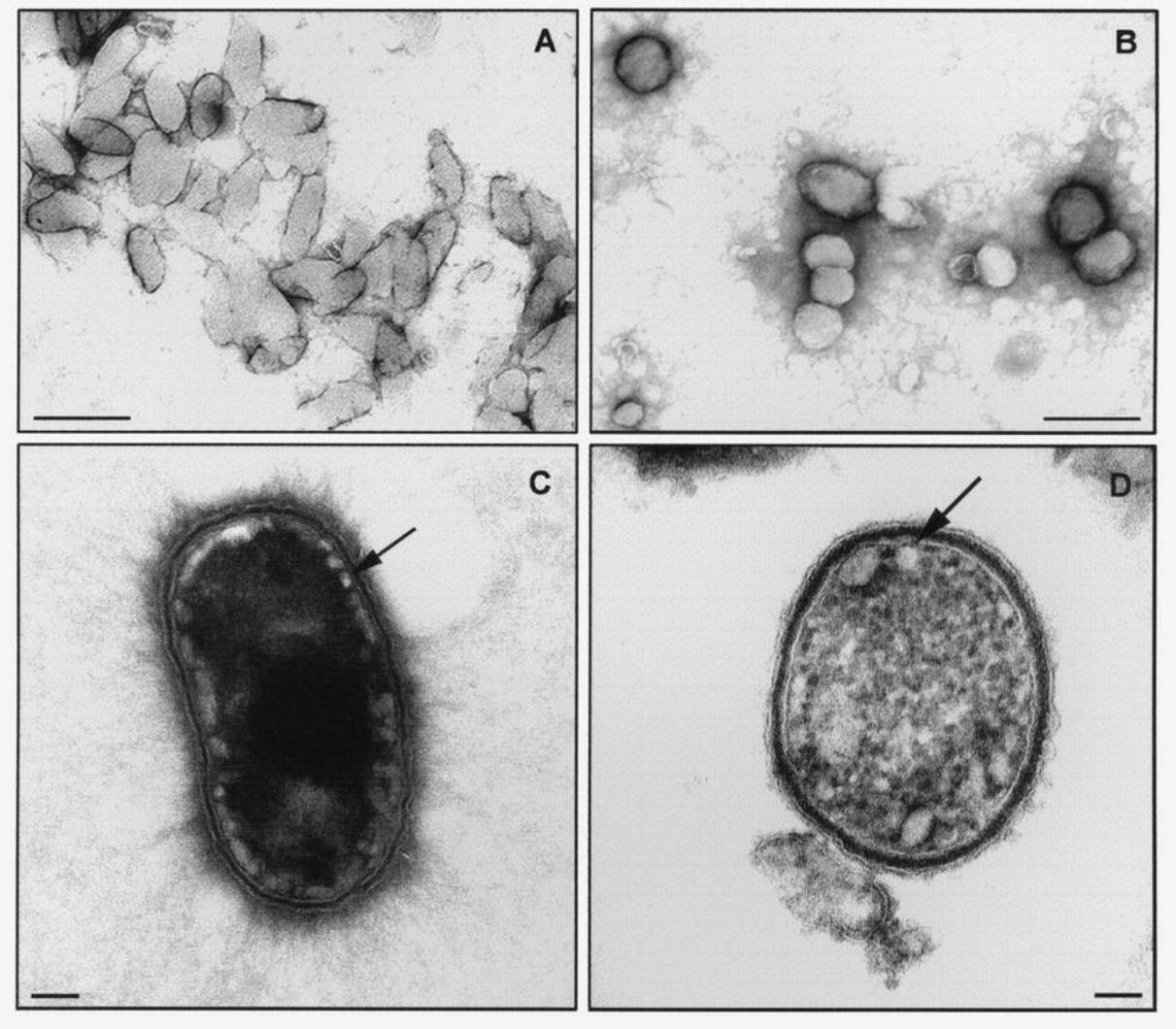

Fig. 4. Electron micrographs of isolated chlorosomes (**A** control, **B** carotenoid-depleted) and cells (**C** control, **D** 2-hydroxybiphenyl-treated) of *Chl. phaeobacteroides* strain CL1401. In ultra-thin frozen sections of cells (**C**, **D**), *arrows* point to chlorosomes attached to the inner face of the cytoplasmic membrane. *Bars* **A**, **B** 200 nm, **C**, **D** 100 nm

Table 2. Effect of HBP treatment on chlorosome dimensions. All values shown are means±standard deviations for n measurements

	Length (nm)	Width (nm)	Height (nm)	Volume ($\times 10^3$ nm^3)
Control (*n*=108)	170±28.2	78.5±15.4	29.4±5.52	205±54.9
Treated (*n*=116)	95.4±18.7	71.9±14.6	54.7±17.8	196±76.5

Discussion

Chemical inhibitors have traditionally been used as an alternative method to inhibit pigment synthesis in the absence of mutagenic molecular techniques (Cohen-Bazire and Stanier *1958*; Kleinig *1974*; Leutwiler and Chapman *1979*; Ormerod et al. *1990*). Foidl et al. (*1997*) and Frese et al. (*1997*) used 2-hydroxybiphenyl as a specific inhibitor of carotenoid biosynthesis in order to determine its effect on phototrophic growth and chlorosome formation in *Cfl. aurantiacus*. Although our work is similar in some respects to these studies, a number of differences should be pointed out: First, the concentration of inhibitor used in this study was three times lower than that used by Foidl et al. (*1997*). This could be due to a difference in the metabolism of the two species, (e.g. *Chlorobium* is a strictly anaerobic photoautotrophic bacterium, while *Chloroflexus* is preferentially photoorganotrophic and can grow aerobically in the dark). Second, in this study carotenoid inhibition caused more noticeable changes in the structure and pigment-protein composition of chlorosomes from *Chl. phaeobacteroides* than those reported in *Cfl. aurantiacus*.

In green sulfur bacteria, the bulk of carotenoids is found in chlorosomes. In particular, in *Chl. phaeobacteroides* more than 80% of the total carotenoids is contained in these vesicles (Borrego et al. *1999*). In the present work, 2-hydroxybiphenyl severely inhibited the specific content of isorenieratene and β -isorenieratene, leading to the accumulation of phytoene as previously described in chlorosomes of *Cfl. aurantiacus* (Foidl et al. *1997*). However, 2-hydroxybiphenyl treatment also resulted in a substantial decrease in the specific content of BChl *e* and BChl *a*. It is worth noting that the 83% decrease in the specific BChl *e* content is consistent with the 73% loss of chlorosomes per µm of cytoplasmic membrane in inhibitor-treated cells.

Likewise, changes in the Csm protein pattern of carotenoid-depleted chlorosomes were small, except for the 60%decrease in CsmA and the increase in the 20-kDa protein. A polypeptide of 19.4 kDa, CsmG, has been found in lighter chlorosomes of *Chl. tepidum* (Vassilieva et al. *1999*) and may be the same as the 20-kDa protein band observed in carotenoid-depleted chlorosomes. The failure to detect CsmB and CsmE in chlorosomes from *Chl. phaeobacteroides* by SDS-PAGE analysis agrees with the earlier results of Van Walree et al. (*1999*) and Stolz et al. (*1990*) and may confirm the evolutionary divergence between chlorosomes from brown- and green-colored sulfur bacteria (Cahill and Stolz *1995*).

In addition to the decrease in the number of chlorosomes, 2-hydroxybiphenyl treatment caused changes in chlorosome morphology and in the arrangement of chlorosomal BChls. Carotenoid-depleted chlorosomes were less homogeneous in size and more rounded. Since the removal of chlorosomal proteins by detergent led to more rounded chlorosomes, Miller et al. (*1993*) suggested that Csm proteins could play a role in maintaining the ellipsoidal shape of these vesicles. In the present study, the partial loss of CsmA might reasonably explain the rounded shape observed in these chlorosomes, since it is the most abundant Csm protein in chlorosomes of *Chl. phaeobacteroides*.

In whole cells, the reduction in the specific content of BChl *a* may be associated with a high sensitivity to 2-hydroxybiphenyl(Foidl et al. *1997*) or with a disorganization of the baseplate caused by carotenoid deficiency. Although the former hypothesis may be correct, it is noteworthy that control and carotenoid-depleted chlorosomes have an apparently equal number of BChl *e* molecules, judging by their mean volumes (Table 2), but they differ in their mean lengths by a factor of two, suggesting that baseplate dimensions in carotenoid-depleted chlorosomes are smaller, as are the baseplate BChl *a* and CsmA contents. Colored carotenoids are known to play a key role in the assembly of functional light-harvesting complexes in purple bacteria and higher plants (Zurdo et al. *1993*; Lang and Hunter

1994; Paulsen *1999*). In green bacteria, this role has also been proposed (Foidl et al. *1997*; Cox et al. *1998*). Moreover, CsmA is the only protein with a conserved possible BChl-binding site, and it could be directly involved in the organization of chlorosomal BChls (Blankenship et al. *1995*; Olson *1998*). An association between BChl *a* and CsmA has been proposed in chlorosomes of *Cfl. aurantiacus* (Sakuragi et al. *1999*). Furthermore, the recent isolation of the B795 baseplate light-harvesting complex of *Cfl. aurantiacus* has clearly shown the molecular association between BChl *a*, CsmA and β -carotene, suggesting that carotenoids play an essential role in the structure and function of this complex (G. Montaño, Arizona State University, Tempe AZ, personal communication). Here, the parallel decreases in BChl *a* and CsmA also support the view that carotenoids play a role in baseplate assembly. Likewise, a smaller baseplate or a lack of carotenoids able to "glue" chlorosomes to the cytoplasmic membrane might explain why chlorosomes detached from the cytoplasmic membrane have sometimes been observed. However, this could also be due to tangential sectioning of the cell surface (Gibson et al. *1984*). It has also been suggested that carotenoids photoprotect BChls in chlorosomes. P š encík et al. (*1994*, *1997*) and Carbonera et al. (*1998*) proposed that carotenoids were irregularly distributed within the chlorosome matrix, most probably close to the baseplate, where excitation may accumulate. Arellano et al. (*2000b*) also suggested that BChl *e* in chlorosomes of *Chl. phaeobacteroides* was less vulnerable to photodamage than BChl *a*. Taken together, these arguments favor the view that carotenoids in chlorosomes are located close to BChl *a* and photoprotect it, while also maintaining the organization of the chlorosome baseplate.

Acknowledgements. This study was funded by the European Community (Contract N0 FMRX-CT96-0081). J.B. Arellano was partly supported by the Spanish Ministry of Education and Culture (Ref. BIO96-1229-002-01). The authors wish to thank the Microscopy Service of the University of Girona and the Research Services of the University of Barcelona for the electron microscopy facilities.

References

Arellano JB, P š encík J, Borrego, CM, Ma Y-Z, Guyoneaud R, Garcia-Gil J, Gillbro T (2000a) Effect of carotenoid biosynthesis inhibition on the chlorosome organization in *Chlorobium phaeobacteroides* strain CL1401. Photochem Photobiol 71: 715-723

Arellano JB, Melø TB, Borrego CM, Garcia-Gil LJ, Naqvi KR (2000b) Nanosecond laser photolysis studies of chlorosomes and artificial aggregates containing bacteriochlorophyll *e*: Evidence for the proximity of carotenoids and bacteriochlorophyll *a* in chlorosomes from *Chlorobium phaeobacteroides* strain CL1401. Photochem Photobiol 72:669-675

Blankenship RE, Olson JM, Miller M (1995) Antenna complexes from green photosynthetic bacteria. In: Blankenship RE, Madigan MT, Bauer CE (eds) Anoxygenic photosynthetic bacteria. Kluwer, Dordrecht, pp 399-435

Borrego CM, Garcia-Gil LJ (1994) Separation of bacteriochlorophyll homologues from green photosynthetic sulfur bacteria by reverse-phase HPLC. Photosynth Res 41:157-163

Borrego CM, Arellano JB, Abellà CA, Gillbro T, Garcia-Gil LJ (1999) The molar extinction coefficient of bacteriochlorophyll *e* and the pigment stoichiometry in *Chlorobium phaeobacteroides.* Photosynth Res 60:257-264

Cahill AD, Stolz JF (1995) Polyclonal antibodies to chlorosome proteins as probes for green sulfur bacteria. Appl Environ Microbiol 61:784-787

Carbonera D, Giacometti G, Vannini C, Gerola PD, Vianelli A, Maniero A, Brunel LC (1998) Electron magnetic resonance of the chlorosomes from green sulfur bacterium *Chlorobium tepidum*. In: Garab G (ed) Photosynthesis: mechanisms and effects, vol I. Kluwer, Dordrecht, pp 109-112

Chung S, Bryant DA (1996) Characterization of the *csmD* and *csmE* genes from *Chlorobium tepidum*. The CsmA, CsmC, CsmD, and CsmE proteins are components of the chlorosome envelope. Photosynth Res 50:41-59

Cohen-Bazire G, Stanier RY (1958) Inhibition of carotenoid synthesis in photosynthetic bacteria. Nature 181:250-252

Connolly JS, Samuel EB, Janzen F (1982) Effects of solvent on the fluorescence properties of bacteriochlorophyll *a*. Photochem Photobiol 36:565-574

Cox RP, Miller M, Aschenbrücker J, Ma YZ, Gillbro T (1998) The role of bacteriochlorophyll *e* and carotenoids in light harvesting in brown-colored green sulfur bacteria. In: Garab G (ed) Photosynthesis: mechanisms and effects, vol I. Kluwer, Dordrecht, pp 149-152

Foidl M, Golecki JR, Oelze J (1997) Phototrophic growth and chlorosome formation in *Chloroflexus aurantiacus* under conditions of carotenoid deficiency. Photosynth Res 54:219-226

Frese R, Oberheide U, van Stokkum I, van Grondelle R, Foidl M, Oelze J, van Amerongen H (1997) The organization of bacteriochlorophyll *c* in *Chloroflexus aurantiacus* and the structural role of carotenoids and protein. Photosynth Res 54:115-126

Frigaard NU, Takaichi S, Hirota M, Shimada K, Matsuura K (1997) Quinones in chlorosomes of green sulfur bacteria and their role in the redox-dependent fluorescence studied in chlorosome-like bacteriochlorophyll *c* aggregates. Arch Microbiol 167:343-349

Gerola PD, Olson JM (1986) A new bacteriochlorophyll *a*-protein complex associated with chlorosomes of green sulfur bacteria. Biochim Biophys Acta 848:69-76

Gibson J, Pfennig N, Waterbury JB (1984) *Chloroherpeton thalassium* gen. nov. et spec. nov., a non-filamentous, flexing and gliding green sulfur bacterium. Arch Microbiol 138:96-101

Griebenow K, Holzwarth AR, van Mourik F, van Grondelle R (1991) Pigment organization and energy transfer in green bacteria. 2. Circular and linear dichroism spectra of protein-free chlorosomes isolated from *Chloroflexus aurantiacus* strain Ok-70-fl. Biochim Biophys Acta 1058:194-202

Holo H, Broch-Due M, Ormerod JG (1985) Glycolipids and the structure of chlorosomes of green bacteria. Arch Microbiol 143:94-99

Holzwarth AR, Schaffner K (1994) On the structure of bacteriochlorophyll molecular aggregates in the chlorosomes of green bacteria. A molecular modelling study. Photosynth Res 41:225-233

Holzwarth AR, Griebenow K, Schaffner K (1990) A photosynthetic antenna system which contains a protein-free chromophore aggregate. Z Naturforsch 45:203-296

Kleinig H (1974) Inhibition of carotenoid synthesis in *Myxococcus fulvus* (Myxobacterales). Arch Microbiol 97: 217-226

Lang HP, Hunter CN (1994) The relationship between carotenoid biosynthesis and the assembly of the light-harvesting LH2 complex in *Rhodobacter sphaeroides*. Biochem J 298:197-205

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature 227:680-685

Leutwiler LS, Chapman DJ (1979) Biosynthesis of aryl carotenoids: inhibition studies of chlorobactene biosynthesis in *Chlorobium limicola* f. *thiosulfatophilum*. Arch Microbiol 123:267-273

Liaaen-Jensen S (1965) Bacterial carotenoids. XVIII. Aryl carotenoids from *Phaeobium.* Acta Chem Scand 19:1025-1030

Lowry OH, Rosebrough NH, Farr AL, Randall RJ (1951) Protein measurements with Folin phenol reagent. J Biol Chem 193:265-275

Melø TB, Frigaard NU, Matsuura K, Naqvi KR (2000) Electronic energy transfer involving carotenoid pigments in chlorosomes of two green bacteria: *Chlorobium tepidum* and *Chloroflexus aurantiacus*. Spectrochim Acta Part A 56:2001-2010

Miller M, Simpson D, Redlinger TE (1993) The effect of detergent on the structure and composition of chlorosomes isolated from *Chloroflexus aurantiacus*. Photosynth Res 35:275-283

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, Dordrecht, pp 259-278

Olson JM (1998) Chlorosome organization and function in green photosynthetic bacteria. Photochem Photobiol 67:61-75

Ormerod JG, Nesbakken T, Beale SI (1990) Specific inhibition of antenna bacteriochlorophyll synthesis in *Chlorobium vibrioforme* by anaesthetic gases. J Bacteriol 172:1352-1360

Otte SCM, van der Heiden JC, Pfennig N, Amesz J (1991) A comparative study of the optical characteristics of intact cells of photosynthetic green sulfur bacteria containing bacteriochlorophyll *c*, *d* or *e*. Photosynth Res 28:77-87

Overmann J, Cypionka H, Pfennig N (1992) An extremely low-light-adapted phototrophic sulfur bacterium from the Black Sea. Limnol Oceanogr 37:150-155

Paulsen H (1999) Carotenoids and the assembly of light harvesting complexes. In: Frank AH, Young AJ, Britton G, Cogdell RJ (eds) The photochemistry of carotenoids. Kluwer, Dordrecht, pp 123-135

PŠencík J, Searle GFW, Hala J, Schaafsma TJ (1994) Fluorescence detected magnetic resonance (FDMR) of green sulfur photosynthetic bacteria *Chlorobium* sp. Photosynth Res 40:1-10

PŠencík J, Schaafsma TJ, Searle GFW, Hala J (1997) Fluorescence detected magnetic resonance of monomers and aggregates of bacteriochlorophylls of green sulfur bacteria *Chlorobium* sp. Photosynth Res 52:83-92

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

Schmidt K (1980) A comparative study on the composition of chlorosomes (*Chlorobium* vesicles) and cytoplasmic membranes from *Chloroflexus aurantiacus* OK-70-fl and *Chlorobium limicola* f. *thiosulfatophilum* strain 6230. Arch Microbiol 124:21-31

Senge MO, Smith KM (1995) Biosynthesis and structures of the bacteriochlorophylls. In: Blankenship RE, Madigan MT, Bauer CE (eds) Anoxygenic photosynthetic bacteria. Kluwer, Dordrecht, pp 137-151

Steensgaard DB, Matsuura K, Cox RP, Miller M (1997) Changes in bacteriochlorophyll c organization during acid treatment of chlorosomes from *Chlorobium tepidum*. Photochem Photobiol 65:129-134

Stolz JF, Fuller RC, Redlinger TE (1990) Pigment-protein diversity in chlorosomes of green phototrophic bacteria. Arch Microbiol 154:422-427

Takaichi S, Wang ZY, Umetsu M, Nozawa T, Shimada K, Madigan MT (1997) New carotenoids from the thermophilic green sulfur bacterium *Chlorobium tepidum*: $1'$,$2'$ -dihydro-γ -carotene,

$1'$,$2'$ -dihydrochlorobactene, and OH-chlorobactene glucoside ester, and the carotenoid composition of different strains. Arch Microbiol 168:270-276

Trüper HG, Pfennig N (1992) The family Chlorobiaceae. In: Balows A, Trüper HG, Dworkin M, Harder W, Schleifer KH (eds) The prokaryotes, 2nd edn. Springer, Berlin Heidelberg New York, pp 3583-3592

Tokuyasu KT (1978) A study of positive staining of ultrathin frozen sections. J Ultrastr Res 63:287-307

Van Dorssen RJ, Vasmel H, Amesz J (1986) Pigment organization and energy transfer in the green photosynthetic bacterium *Chloroflexus aurantiacus.* II. The chlorosome. Photosynth Res 9:33-45

Van Walree CA, Sakuragi Y, Steensgaard DB, Bösinger 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

Vassilieva EV, Antonkine ML, Zybailov BL, Yang F, Jakobs CU, Golbeck JH, Bryant DA (2001) Electron transfer may occur in the chlorosome envelope: the csmi and csmj proteins of chlorosomes are 2fe-2s ferredoxins. Biochemistry 4:464-473

Wagner-Huber R, Brunisholz R, Frank G, Zuber H (1988) The bacteriochlorophyll *c*/*e*- binding polypeptides from chlorosomes of green photosynthetic bacteria. FEBS Lett 239:8-12

Wullink W, Knudsen J, Olson JM, Redlinger TE, van Bruggen EFJ (1991) Localization of polypeptides in isolated chlorosomes from green phototrophic bacteria by immunogold labelling electron microscopy. Biochim Biophys Acta 1060:97-105

Zurdo J, Fernández-Cabrera C, Ramírez JM (1993) A structural role of the carotenoid in the light-harvesting II protein of *Rhodobacter capsulatus*. Biochem J 290:531-537