Biochimica et Biophysica Acta 848 (1986) 69–76
Elsevier

BBA 41906

A new bacteriochlorophyll *a*-protein complex associated with chlorosomes of green sulfur bacteria

Paolo D. Gerola * and John M. Olson **

Institute of Biochemistry, Odense University, Campusvej 55, 5230 Odense M (Denmark)

(Received July 2nd, 1985)

Key words: Bacteriochlorophyll *a*; Chlorosome; Chlorophyll-protein complex; (Green sulfur bacterium)

Chlorosomes were prepared from *Chlorobium limicola* f. *thiosulfatophilum* by sucrose density gradient centrifugation. Cells broken in the presence of 2 M NaSCN yielded three chlorosome fractions in the gradient: low density (no sucrose), medium density (approx. 18% sucrose), and high density (approx. 26% sucrose). All fractions were stable at any chlorosome concentration. Cells broken in the absence of 2 M NaSCN also yielded three fractions, but only the high-density fraction contained stable chlorosomes. The medium-density chlorosomes were stable only when highly concentrated. Upon dilution, bacteriochlorophyll (BChl) *c* was degraded to bacteriopheophytin *c* and concomitantly a band at 794 nm (BChl *a*) was revealed. Two 794-nm fractions were observed with the same densities as low- and medium-density chlorosomes. The protein composition of the 794-nm fractions was similar to that of the stable chlorosome fractions. All showed a 4–5 kDa (M_r) protein as a major component, but no trace of the 40-kDa protein characteristic of the water-soluble BChl *a*-protein of green sulfur bacteria. BChl *a* was present in all types of chlorosomes, in stable chlorosomes the BChl *c*/BChl *a* ratio was approx. 90. A special BChl *a*-protein (794 nm) inside the chlorosome is postulated to mediate energy transfer from BChl *c* to the water-soluble BChl *a*-protein in the baseplate.

Introduction

Photosynthetic green bacteria are classified in two groups, the Chlorobiaceae and the Chloroflexaceae, which are quite far apart from an evolutionary point of view [1]. They also are physiologically quite different. The Chlorobiaceae are strictly anaerobic photoautotrophic bacteria, while the Chloroflexaceae can grow either photo-organotrophically or chemo-organotrophically in the dark depending on the oxygen tension and the light intensity. The two reaction centers are also very different. The Chloroflexaceae are characterized by a PS-II-like reaction center, physiologically similar to that present in the purple bacteria, while the Chlorobiaceae contain a PS-I-like reaction center able to reduce ferredoxin directly from a low potential Fe-S protein in the reaction center [2]. In spite of these fundamental differences, both Chloroflexaceae and Chlorobiaceae contain BChl *c* or a similar pigment as the main light-harvesting pigment. BChl *c* is localized inside oblong bodies called chlorosomes, which are appressed to the inner surface of the cytoplasmic membrane where the reaction centers are localized [3]. Much effort has been spent in the last few years to investigate the composition and organization of chlorosomes

* Present address: Sezione Fisiologia Vegetale-Fotosintesi, Dipartimento di Biologia, Università di Milano, Via Celoria 26, Milano, Italy.
** To whom correspondence should be addressed.

Abbreviations: BChl, bacteriochlorophyll; BPh, bacteriopheophytin; CD, circular dichroism; PMFS, phenylmethanesulfonylfluoride; SDS, sodium dodecyl sulfate.

and also to study energy transfer between the BChl *c* and the reaction centers [4–12]. Electron microscope studies have revealed that inside the chlorosomes of both Chloroflexaceae and Chlorobiaceae there are rod elements extending the full length of the chlorosomes [5,7]. In the Chlorobiaceae the 10-nm diameter rod elements appear to contain 15-kDa proteinacious subunits, each of which is associated with 12–14 BChl *c* molecules [3,4]. In the Chloroflexaceae the 5.2-nm diameter rod elements appear to consist of 5.6-kDa protein subunits, each associated with seven BChl *c* molecules [12]. In both Chloroflexaceae and Chlorobiaceae the chlorosomes appear to be bounded by a monolayer of lipid [5,7], mainly monogalactosyldiacylglycerols [4,6].

The chlorosomes of Chloroflexaceae contain a special form of BChl *a* (Q_y-band at 792 nm) possibly associated with a 5.8-kDa (M_r) protein [6,12]. From energy-transfer studies [10] it appeared that the BChl *a*-protein facilitates the transfer of excitation energy from BChl *c* inside the chlorosome to BChl *a*-protein complexes and reaction centers in the cytoplasmic membrane. Although Schmidt [6] detected some BChl *a* in purified chlorosome fractions from *Chlorobium,* it might have been due to contamination by the water-soluble BChl *a*-baseplate protein. In this paper we demonstrate that inside the Chlorobiacean chlorosome there is a BChl *a*-protein with Q_y-band at 794 nm, and that the Chlorobiacea chlorosome is organized more like the Chloroflexacea chlorosome than was previously realized.

Materials and Methods

Chlorosome preparation

Chlorobium limicola f. *thiosulfatophilum* 6230 (Tassajara) was grown in batch culture as described previously [13]. The bacteria were harvested and resuspended in 10 mM P_i/10 mM ascorbate, pH 7.4 buffer (4 ml/g wet weight of bacteria) with a Potter homogenizer. They were then passed 3 × through an ice-cold French pressure cell at 110 MPa. After the first passage they were incubated at room temperature for 30 min in the presence of DNAase. The broken cells were centrifuged at 18000 × *g* for 20 min to remove unbroken cells and large debris. The supernatant was then centrifuged again at 48000 × *g* for 90 min, and the pellet was resuspended in buffer. The 48000 × *g* supernatant, although still containing considerable amounts of chlorosome material, was enriched in membrane fragments, whereas the pellet was enriched in chlorosomes. Both supernatant and resuspended precipitate were loaded onto 20–50% (w/w) sucrose gradients (all sucrose gradients were prepared in buffer). These gradients, named 'supernatant' and 'pellet' preparative gradients, respectively, were centrifuged approx. 18 h at 5°C in a SB283 swinging bucket rotor (B-60 International ultracentrifuge) at 240000 × *g*. The main chlorosome band was present in the higher part of the gradient, while membrane fragments banded at about 40% sucrose. Two bands characterized by the presence of a 794-nm peak were observed, one at the top of the buffer, the other one between buffer and the sucrose gradient (see Results). When 2 M NaSCN was present during chlorosome preparation, the centrifugation at 48000 × *g* was omitted. After the centrifugation at 18000 × *g* two pellets were present, one at the bottom of the tube (unbroken cells, large debris) and one at the top (low-density material). The supernatant and the pellet on the top were collected together and loaded onto the preparative gradient (10–50% w/w sucrose in buffer + 2 M NaSCN). The gradient was centrifuged as in the absence of NaSCN. Two main chlorosome bands were observed, one at the top of the 2 M NaSCN alone and the other one in the higher part of the sucrose gradient.

Chlorosome and 794-nm fraction purification

Sucrose (20% w/w) was added to increase the density of the enriched fractions. They were then loaded onto a small two-step (40%, 50% w/w) sucrose gradient, and a continuous sucrose gradient was loaded over the sample. Finally, a small amount of buffer was added at the top. The range of the continuous sucrose gradient varied according to the density of the sample which was to be purified. These purification gradients were run under the same conditions as the preparative

According to our usage, the word 'baseplate' refers only to the 2-dimensional crystals of water-soluble BChl *a*-protein found in Chlorobiaceae. Baseplates thus defined are not present in Chloroflexaceae.

gradients. When necessary, another gradient was run either for purification or concentration of the sample.

Polyacrylamide gel electrophoresis

This procedure was carried out according to Laemmli [14] with a few modifications. 1-mm thick gels were used. Stacking gel was 6% acrylamide; running gel, 16% acrylamide or 12–20% acrylamide gradient. Samples were heated at 60°C for 30 min in 0.5% SDS/10% mercaptoethanol/10% glycerol/62.5 mM Tris (pH 6.8) in the presence of Bromophenol blue. Gels were run at a fixed current, starting at a voltage of 90 V for 6–8 h until the Bromophenol blue reached the bottom of the gel. Gels were fixed overnight in 45% methanol/9.5% acetic acid/5% formaldehyde/0.05% Coomassie blue. After destaining they were restained with silver according to Merril et al. [15] with slight modifications. Gels were incubated for 5 min in 5.7 mM $K_2Cr_2O_7$/2 mM HNO_3 (150 ml per gel) and washed three times with water (250 ml per gel, 5 min each washing). They were then incubated for 30 min in 0.98 mM $AgNO_3$ (150 ml/gl) and washed for 30 s with 250 ml 75 mM Na_2CO_3/0.67 mM formaldehyde and developed with 275 ml 0.75 M Na_2CO_3/6.7 mM formaldehyde. After the color was developed the gels were placed in 5% acetic acid.

Absorption spectra were recorded with a Perkin-Elmer Spectrophotometer Model 330, and circular dichroism spectra were recorded with an ISA Jobin Yvon Dichrograph (1975).

Results

Chlorosome preparation

When chlorosomes were prepared in the absence of SCN^- (see Materials and Methods) a new component with a far-red absorption peak at 794 nm was observed. In Fig. 1 the profiles of the 794-nm band and the chlorosomes in the supernatant preparative gradient are shown. The 794-nm band appeared in two fractions, one at the top of the buffer and the other between buffer and 20% sucrose. The presence of chlorosome material in the various fractions of the gradient was monitored at the Q_y-peak of aggregated BChl *c*. Usually no chlorosomes were present at the top of the

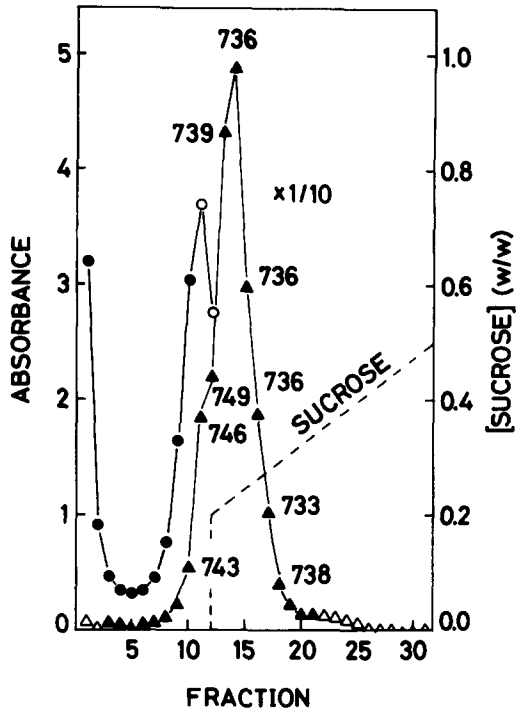

Fig. 1. Profile of the 794-nm band and of the chlorosomes (×1/10) in the supernatant preparative gradient. Chlorosomes were prepared in buffer (see Materials and Methods). ▲, △, chlorosomes, detected as absorbance at the Q_y-peak of BChl *c*. Closed symbols indicate the presence of a BChl *c* Q_y peak, while open symbols indicate the absorbance measured at 745 nm, no BChl *c* Q_y peak being detectable. ●, ○, 794-nm component. Closed symbols indicate the presence of a peak at 794 nm, while open symbols indicate the presence of only a shoulder at 794 nm on the BChl *c* Q_y peak.

buffer; the main chlorosome fraction was found at about 26% sucrose. As can be seen from Fig. 1, the chlorosome profile showed a shoulder between buffer and 20% sucrose. The chlorosomes banding at about 26% sucrose and those responsible for the shoulder in the preparative gradient profile will be called 'high density' chlorosomes and 'medium density' chlorosomes, respectively. Interestingly, the Q_y-peak of BChl *c* in medium-density chlorosomes was shifted farther to the red (747–750 nm) than in high-density chlorosomes (730–745 nm).

A component with an absorption band at about 673 nm was associated with all the fractions (794-nm band, chlorosomes, and membrane fragments) of the gradient. Treatment with 10-mM sodium dithionite bleached this peak. From the absorption

spectrum and the changes in the absorption spectra of the different fractions after dithionite treatment, the component giving rise to the 673-nm band (Fig. 4) was identified as BPh *c*. The different components found in the supernatant-preparative gradient were also observed in the pellet-preparative gradient.

When the chlorosome stability upon storage was checked, it appeared that, while both concentrated and dilute high-density chlorosomes were quite stable, medium-density chlorosomes were stable only at high concentration ($A_{750} > 200$). Upon dilution both the Q_y-peak and the 456-nm peak bleached; there was a slight increase in the 673-nm band, and a band at 794 nm became visible (see Fig. 2). The rate of this degradation process was increased by treatment of the diluted medium density chlorosomes with 0.05% (v/v) Miranol. A similar chlorosome instability was reported by Cruden and Stanier [4]. Different conditions of osmolarity, pH, redox environment, [$MgCl_2$], [PMFS], and [NaSCN] were tested to increase the stability of medium-density chlorosomes, but the only effective one was treatment with 2 M NaSCN. The chlorosomes were then prepared in the presence of 2 M NaSCN (see Materials and Methods), and the amount of BPh *c* in the preparation appeared greatly reduced.

No 794-nm fraction was observed in the preparative gradient in the presence of 2 M NaSCN,

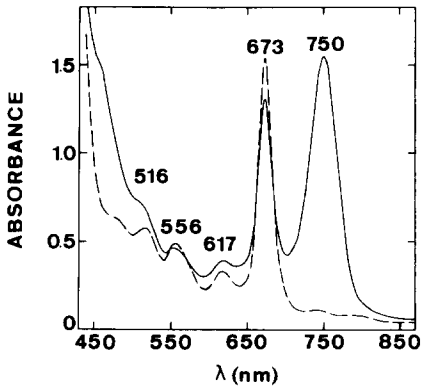

Fig. 2. Medium-density chlorosome instability. Chlorosomes were prepared in buffer (see Materials and Methods). Continuous line, absorbance spectrum after five months of storing the chlorosomes frozen at high concentration ($A_{750} = 340$). Dashed line, absorbance spectrum after 24 h of storing diluted (A_{750}, 1.56) chlorosomes at room temperature.

and the chlorosomes were concentrated in two main fractions: the major one in the upper part of the sucrose gradient and the other one at the top of the 2 M NaSCN in P_i buffer (density about that of 23% sucrose) (see Fig. 3A). This second fraction, with lower density, was characterized by a Q_y-peak (747–749 nm) more shifted to the far-red than was the one (740–745 nm) at higher density. Chlorosomes were also present at a very low concentration in the higher-density regions of the gradient (see Fig. 3A).

When the two main chlorosome fractions were further purified on sucrose gradients (see Materials and Methods), the higher density fraction gave rise to a chlorosome band at about 25% sucrose (high-density chlorosomes) (data not shown), while the lower density fraction gave rise to two different chlorosome bands: a major one at about 18% sucrose (medium-density chlorosomes) and a minor one on the top of the buffer (low-density chlorosomes) (see Fig. 3B). Both medium- and low-density chlorosomes showed the same Q_y-peak at 747–749 nm. Since each chlorosome fraction reached the same final position in both 'preparative' and 'purification' gradients, in which the migration was in opposite directions (see Materials and Methods), it was clear that the final position was based on the density rather than on the migration rate.

794-nm fraction characterization

The 794-nm fraction was further purified as described in Materials and Methods. The absorption spectrum is shown in Fig. 4. The peaks at 672, 613, 552 and 416 nm were probably due to BPh *c*, while carotenoids were probably responsible for those at 473 and 506 nm. The absorption spectrum of the 90% methanol extract (Fig. 4) indicated that the 794-nm peak was due to BChl *a*. The CD spectrum of the purified fraction is shown in Fig. 5. A very small negative signal was associated with the 673-nm absorption peak due to monomeric BPh *c* (data not shown), while a larger conservative CD signal ($\Delta A_{792} - \Delta A_{812} = 7.9 \cdot 10^{-4}$) was associated with the 794-nm absorbance peak ($A = 1.0$). Gel electrophoresis of the sample (Fig. 6A) showed only one band at about 4–5 kDa (M_r) after Coomassie brilliant blue staining. Silver staining revealed the presence of three other minor

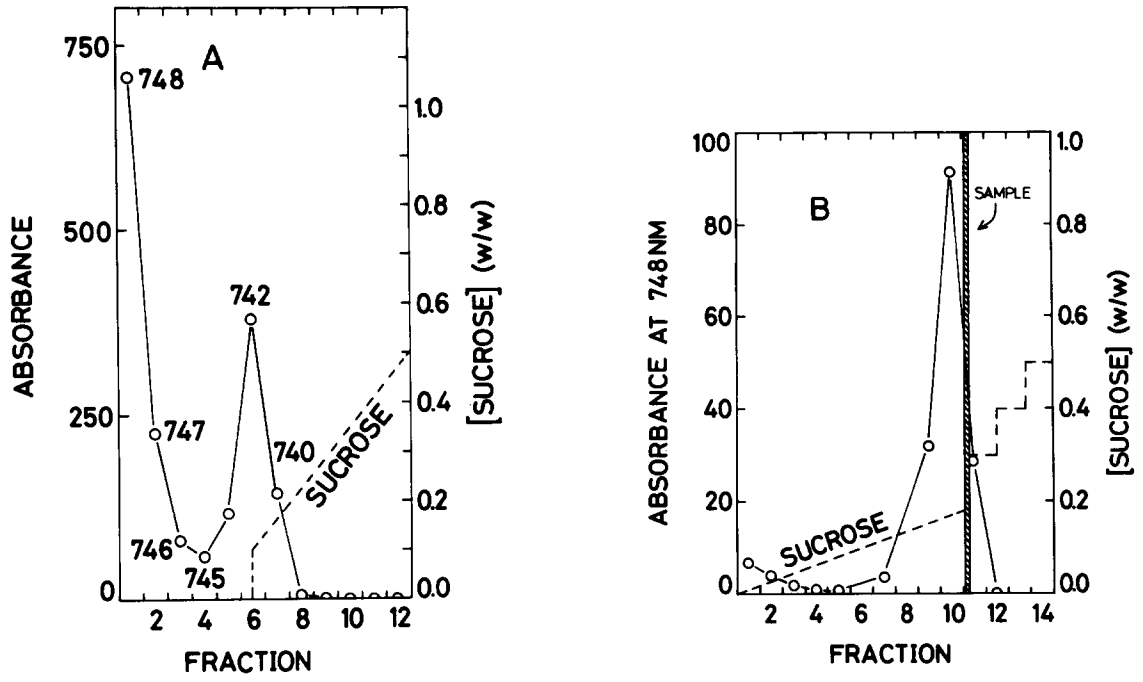

Fig. 3. (A) Profile of the chlorosome preparative gradient. Chlorosomes were prepared in the presence of 2 M NaSCN (see Materials and Methods). (B) Profile of the purification gradient of the lower-density chlorosome fraction (see Materials and Methods). Chlorosomes were detected by their absorbance at the BChl *c* Q_y peak (748 nm).

bands with relative molecular weights (M_r) of about 26, 36 and 43 kDa.

Chlorosome characterization

Low-, medium- and high-density chlorosomes were further purified as described in Materials and Methods. In Fig. 7 are shown the absorption spectra of high-density chlorosomes and their acetone-methanol extract. The 456- and 744-nm peaks are due to BChl *c*, the 672-nm peak is probably due to BPh *c*, and the shoulder at 510 nm is due to carotenoids. BChl *c* (and possibly BPh *c*) contribute to the 334- and the 428-nm peaks.

Table I summarizes the BChl *c*/Chl *a* molar

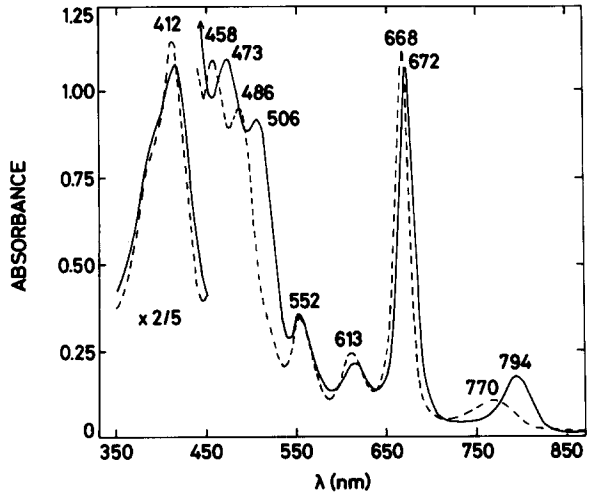

Fig. 4. Absorbance spectrum of the purified 794-nm fraction (continuous line) and of its 90% methanol extract (dashed line).

TABLE I

BChl *c*/BChl *a* MOLAR RATIOS IN DIFFERENT PREPARATIONS OF PURIFIED CHLOROSOMES

BChl *c*/BChl *a* molar ratios were estimated both in 90% methanol extracts and in H_2O/acetone/methanol (1 : 19.4 : 5.6) extracts. A ratio of 0.927 between the extinction coefficients for BChl *a* and for BChl *c* was used, according to Ref. 11.

Preparation	Low	Medium	High
1	82	87	104
2 } 2 M NaSCN	46	86	89
3	–	46	56
4 buffer	–	–	87

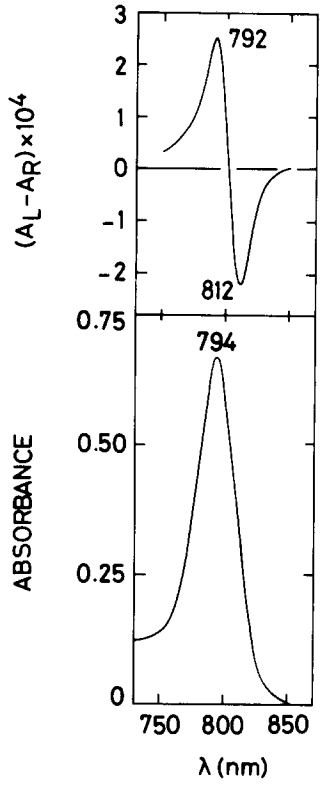

Fig. 5. Absorbance and CD spectrum of the 794-nm component (see Materials and Methods).

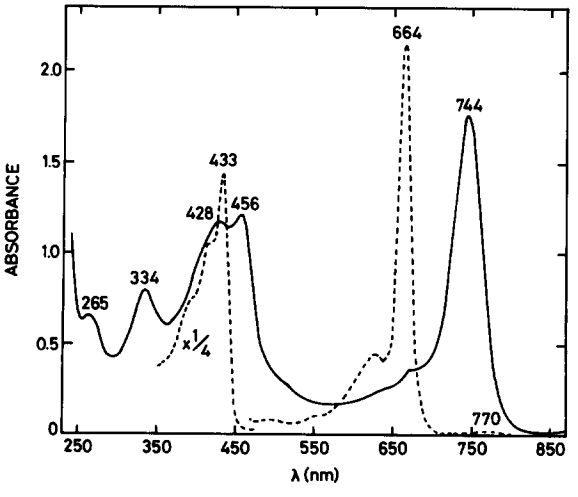

Fig. 7. Absorbance spectra of purified high-density chlorosomes prepared in the presence of 2 M SCN (continuous line) and of their extract (dashed line) in H_2O/acetone/methanol (1 : 19.4 : 5.6).

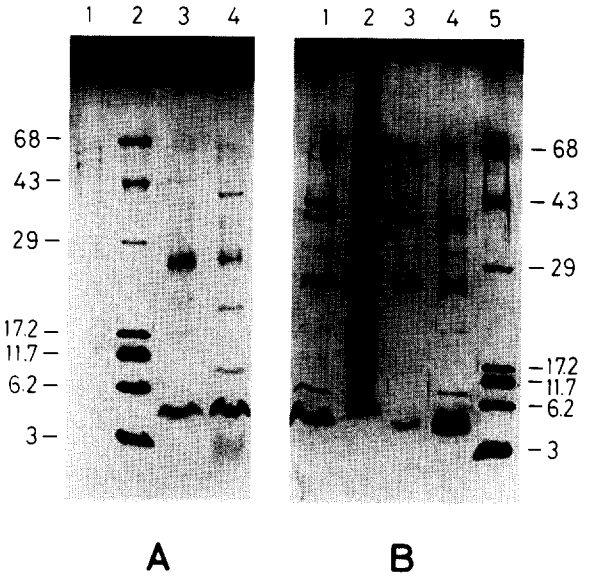

Fig. 6. Polyacrylamide gel electrophoresis of 794-nm fraction, high density chlorosomes, membrane fragments and water soluble BChl *a*-protein (808 nm). (A) Lane 1, solubilization buffer; lane 2, standards; lane 3, 794-nm fraction (A_{794}(loaded) = 0.01); lane 4, high-density chlorosomes (A_{744}(loaded) = 1). Running gel, 16% acrylamide. (B) Lane 1, chlorosomes (A_{744} (loaded) = 1) plus water soluble BChl *a* protein (A_{808} (loaded) = 0.0063); lane 2, membrane fragments (A_{810} (loaded) = 0.0018); lane 3, high-density chlorosomes (A_{744} (loaded) = 0.53); lane 4, high-density chlorosomes (A_{744} (loaded) = 1); lane 5, standards. Running gel, 12–20% acrylamide gradient. The following standards were used: bovine serum albumin (68 kDa, 0.4 μg), ovalbumin (43 kDa, 0.4 μg), carbonic anhydrase (29 kDa, 0.4 μg), myoglobin (17.2 kDa, 0.4 μg), cytochrome *c* (11.7 kDa, 0.4 μg), aprotinin (6.2 kDa, 0.4 μg) and insulin (3 kDa, 0.68 μg). Running, fixing and staining conditions, see Materials and Methods.

ratios measured in low-, medium- and high-density chlorosomes from four different preparations. From the comparison of the different chlorosomes obtained from the same preparations it appears that the medium- and high-density chlorosomes had similar BChl *c*/BChl *a* ratios, while lower values could sometimes be observed in the low-density chlorosomes, probably due to their partial degradation. A certain variability was observed in low-, medium- or high-density chlorosomes obtained from different preparations, but a ratio of about 90 BChl *c*/BChl *a* molecules was usually found.

Polyacrylamide gel electrophoresis indicated

that all three kinds of chlorosomes have the same protein composition (data not shown). A major band was present at approx. 4–5 kDa (M_r), and it was the only one observed after Coomassie brilliant blue staining. Silver staining revealed the presence of six other bands at about 9, 20, 26, 27, 32 and 38 kDa (M) (Fig. 6A and B). There was some variability in the degree of staining of these six minor bands. In Fig. 6B the gel pattern for chlorosomes is compared to that for pure water-soluble BChl *a*-protein (808 nm) and that for membrane fragments.

Membrane fragments

Membrane fragments obtained in the preparative gradient in the absence of SCN^- were different from those obtained in its presence, because SCN^- causes the release of the water soluble BChl *a*-protein (808 nm) located on the surface of the cytoplasmic membrane (data not shown). Denaturation of this BChl *a*-protein was also observed, the extent varying in the different preparations.

Discussion

When chlorosomes are prepared in the presence of 2 M NaSCN, they appear in three different density fractions, low, medium and high density. It is not clear how SCN^- stabilizes these chlorosomes during preparation, but they remain fairly stable in the absence of SCN following their preparation in its presence. The action of SCN^- might be to inactivate some lipase or proteolytic enzyme present in the cell extract. Without an electron microscopic study it is difficult to explain the different densities of the three chlorosome fractions, since they appear to have the same protein composition. Schmidt [6] also observed two different density fractions which might correspond to our medium and high-density chlorosomes. By electron microscopy she observed a contamination of the lighter fraction by a network of threads, probably originating from the capsule of the outer cell layers. The same situation might apply to our medium-density chlorosomes. However, there seems to be an intrinsic difference between low- and medium-density chlorosomes on the one hand and high density chlorosomes on the other. The absorption maximum of the Q_y-band is blue-shifted in high-density chlorosomes; and the rotational strengths are higher than in low- and medium-density chlorosomes (Olson, J.M. and Gerola, P.D., unpublished results).

The 794-nm component is a form of BChl *a* thought to be associated with one of the chlorosome proteins. We have demonstrated that in intact chlorosomes about 1% of the total chlorophyll is BChl *a* and that this BChl *a* cannot be attributed to contamination by the water soluble BChl *a*-protein (808 nm) or membrane fragments. We have further shown that at room temperature the 794-nm form of BChl *a* can be observed spectrophotometrically only in unstable chlorosomes in the absence of 2 M NaSCN. Under these conditions the peak of aggregated BChl *c* near 750 nm disappears, and the 794-nm band of BChl *a* is revealed. The conservative CD spectrum associated with the 794-nm band is distinctly different from that associated with the 808-nm band of the baseplate BChl *a*-protein and indicates a strong interaction between two or more BChl *a* molecules. The BChl *a*-'protein' (794 nm) appears to be a structural part of the chlorosome in *C. limicola* in analogy to the BChl *a*-protein (792 nm) in the

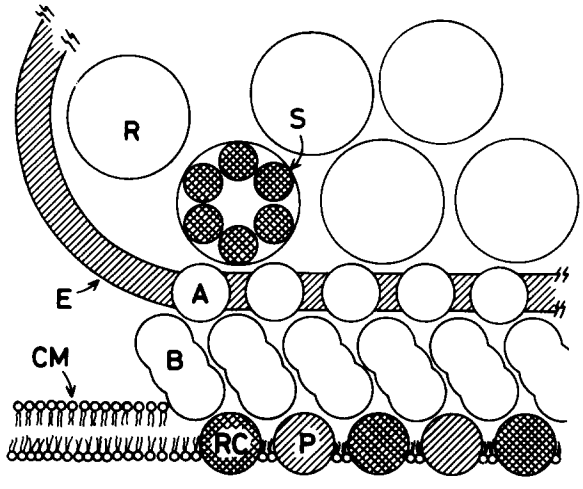

Fig. 8. Model of a chlorosome and associated cytoplasmic membrane (CM) in *C. limicola* f. *thiosulfatophilum*. A, BChl *a*-chlorosome protein; B, BChl *a*-baseplate protein; E, chlorosome envelope; P, pigment complex; R, rod element; RC, reaction center complex; S, 15-kDa subunit is BChl *c*-antenna protein

chlorosome of *Chloroflexus aurantiacus* [6]. In our model for *C. limicola* (Fig. 8) the chlorosome is bounded completely by a monolayer of lipid with the BChl *a*-'protein' (794 nm) localized in the monolayer in a way similar to that proposed for *Chloroflexus* [12]. The chlorosome makes contact with the water-soluble BChl *a*-protein (809 nm) in the baseplate, and the excitation energy from the BChl *c* is transferred to the reaction centers in the cytoplasmic membrane via the BChl *a*-'protein' (794 nm) in the chlorosome monolayer and the water-soluble BChl *a*-protein (808 nm) in the baseplate. Further evidence supporting this model comes from a study of energy transfer between BChl *c* and BChl *a*-'protein' (794 nm) in purified chlorosomes [16].

Acknowledgements

Prof. Norbert Pfennig (University of Constance) provided us with a pure culture of *C. limicola* f. *thiosulfatophilum*, and Prof. Erik Larsen (Royal Veterinary and Agricultural College, Copenhagen) made available the dichrograph for CD measurements. Prof. Jan Amesz (State University of Leiden) made several helpful suggestions about the manuscript. The research was supported by Grant GM 3210 from the United States National Institute of General Medical Sciences.

References

1 Fox, G.E., Stackebrandt, E., Heyell, R.B., Gibson, J., Maniloff, J., Dyer, T.A., Wolfe, R.S., Balch, W.E., Tanner, R.S., Magrum, L.J., Zablen, L.B., Blakemore, R., Gupta, R., Bonen, L., Lewis, B.J., Stahl, D.A., Luehrsen, K.R., Chen, K.N. and Woese, C.R. (1980) Science 109, 457–463
2 Amesz, J. and Knaff, D.B. (1985) in Environmental Microbiology of Anaerobes (A) (Zehnder, A.B., ed.), John Wiley, New York, in the press
3 Olson, J.M. (1980) Biochim. Biophys. Acta 594, 33–51
4 Cruden, D.L. and Stanier, R.Y. (1970) Arch. Mikrobiol. 72, 115–134
5 Staehelin, L.A., Golecki, J.R., Fuller, R.C. and Drews, G. (1978) Arch. Mikrobiol. 119, 269–277
6 Schmidt, K. (1980) Arch. Mikrobiol. 124, 21–31
7 Staehelin, L.A., Golecki, J.R. and Drews, G. (1980) Biochim. Biophys. Acta 589, 30–45
8 Sprague, S.G., Staehelin, L.A., Di Bartolomeis, M.J. and Fuller, R.C. (1981) J. Bacteriol. 147, 1021–1031
9 Sprague, S.G., Staehelin, L.A. and Fuller, R.C. (1981) J. Bacteriol. 147, 1032–1039
10 Betti, J.A., Blankenship, R.E., Natarajan, L.V., Dickinson, L.C. and Fuller, R.C. (1982) Biochim. Biophys. Acta 680, 194–201
11 Feick, R.G., Fitzpatrick, M. and Fuller, R.C. (1982) J. Bacteriol. 150, 905–915
12 Wechsler, T., Suter, F., Fuller, R.C. and Zuber, H. (1985) FEBS Lett. 181, 173–178
13 Olson, J.M., Philipson, K.D. and Sauer, K. (1973) Biochim. Biophys. Acta 292, 206–217
14 Laemmli, U.K. (1970) Nature 227, 680–685
15 Merril, C.R., Goldman, D., Sedman, S.A. and Ebert, M.H. (1981) Science 211, 1437–1438
16 Van Dorssen, R., Gerola, P.D., Olson, J.M. and Amesz, J. (1986) Biochim. Biophys. Acta 848, 77–82