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Association of bacteriochlorophyll *a* with the CsmA protein in chlorosomes of the photosynthetic green filamentous bacterium *Chloroflexus aurantiacus*

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Abstract

The protein assumed to be associated with bacteriochlorophyll (BChl) *a* in chlorosomes from the photosynthetic green filamentous bacterium *Chloroflexus aurantiacus* was investigated by alkaline treatment, proteolytic digestion and a new treatment using 1-hexanol, sodium cholate and Triton X-100. Upon alkaline treatment, only the 5.7 kDa CsmA protein was removed from the chlorosomes among six proteins detected by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis, concomitantly with the disappearance of BChl *a* absorption at 795 nm. Trypsin treatment removed two proteins with molecular masses of 11 and 18 kDa (CsmN and CmsM), whereas the spectral properties of BChl *a* and BChl *c* were not changed. By the new hexanol-detergent (HD) treatment, most BChl *c* and all of the detected proteins except CsmA were removed from the chlorosomes without changing the BChl *a* spectral properties. Subsequent proteinase K treatment of these HD-treated chlorosomes caused digestion of CsmA and a simultaneous decrease of the BChl *a* absorption band. Based on these results, we suggest that CsmA is associated with BChl *a* in the chlorosomes. This suggestion was supported by the measured stoichiometric ratio of BChl *a* to CsmA in isolated chlorosomes, which was estimated to be between 1.2 and 2.7 by amino acid analysis of the SDS-PAGE-resolved protein bands.

Keywords: Bacteriochlorophyll *a*; Baseplate; Chlorosome; Green bacterium; Pigment-protein complex; Protein purification

1. Introduction

The photosynthetic green filamentous bacterium *Chloroflexus aurantiacus* contains light-harvesting antennas called chlorosomes which are attached to the cytoplasmic membrane in which photosynthetic reaction centers, additional light-harvesting complexes (absorbing at 808 and 866 nm) are embedded [1,2]. Photosynthetic green sulfur bacteria also have chlorosomes which are attached to the cytoplasmic membrane via Fenna-Matthews-Olson (FMO) proteins [1,3].

The chlorosomes are oblong organelles in which thousands of bacteriochlorophyll (BChl) *c, d* or *e* are organized in rod-like structures and enveloped by a protein-lipid monolayer. A minor amount of BChl *a* is also present in the chlorosome [4,5], which

Abbreviations: BChl, bacteriochlorophyll; CBB, Coomassie brilliant blue R-250; FMO protein, Fenna-Matthews-Olson protein; HD treatment, hexanol-detergent treatment; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis

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mediates energy transfer to the cytoplasmic membrane [6,7,8]. This BChl *a* is thought to be located in a so-called baseplate which has been observed in the chlorosomes of *C. aurantiacus* as a fine periodical structure by electron microscopy [9] and probably constitutes the attachment site of the chlorosome to the cytoplasmic membrane or to the FMO protein.

Three proteins, CsmA, CsmN and CsmM, are found in isolated chlorosomes from *C. aurantiacus* with apparent molecular weights of 3.7, 11 and 18 kDa based on their relative mobilities in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) [10]. The amino acid sequence of CsmA has been chemically determined [11]. The true mass is found to be 5.7 kDa, which is in agreement with the deduced mass from the sequence of the *csmA* gene encoding this protein [12]. These chlorosomal proteins are located in the lipid envelope, as demonstrated by gold labelling electron microscopy [13]. CsmA in chlorosomes of green sulfur bacteria has a mass of 6.3 kDa and shows 30% homology with CsmA from *C. aurantiacus* [14,15]. The *csmA* gene encoding this 6.3 kDa CsmA has been cloned and sequenced from both *Chlorobium tepidum* and *Chlorobium vibrioforme* [16].

Some researchers have suggested that the CsmA protein is necessary for the BChl *c* organization in chlorosomes of *C. aurantiacus* [10,11,17–19] and the green sulfur bacteria [14,15]. This was mostly based on that they are the most abundant proteins in the chlorosomes and the characteristic absorption band of BChl *c* around 740 nm is significantly decreased following proteolytic digestion of this protein in *C. aurantiacus*. However, this suggestion has been refused by the currently accepted view that BChl *c* in chlorosomes is organized by self-aggregation without proteins. This view has been supported by the observation that all proteins can be removed from chlorosomes with SDS treatment without changing the 740 nm absorption band [20] and the demonstration that BChl *c* can aggregate spontaneously to form a 740 nm band in organic solvents such as hexane or tetrachloromethane [1,21] and aqueous buffers in the presence of detergents or lipids [22–24]. Therefore, the actual role of CsmA in chlorosomes has yet to be revealed.

The Q_y absorption band of BChl *a* in the chlorosome has a maximum at 795 nm, which is 25 nm red-shifted compared to that of the monomeric form of this pigment in methanol. It is most likely that the BChl *a* is organized on a protein scaffold in the chlorosomal baseplate. An additional protein identified in *C. aurantiacus* with an apparent mass of 5.8 kDa has been suggested to serve this function [10]. However, this suggestion has been questioned by Lehmann et al. [19]. In addition, Foidl et al. [25] concluded that there is no correlation between the amount of the 5.8 kDa protein and BChl *a* present in the chlorosomes.

We have studied the role of proteins with respect to the chlorophyll organization in the chlorosomes of *C. aurantiacus*. Isolated chlorosomes from *C. aurantiacus* strain J-10-fl were subjected to proteolytic digestion, alkaline treatment or a newly developed method using hexanol, sodium cholate and Triton X-100. We conclude that BChl *a* is associated with, and possibly binds to, the CsmA protein in *C. aurantiacus*.

2. Materials and methods

2.1. Chlorosome preparation

The green filamentous bacterium *C. aurantiacus* J-10-fl was grown in PE medium [26] at 55°C for about 60 h, harvested by centrifugation at $6000 \times g$ and washed once with a 50 mM Tris-HCl buffer pH 8.6. A modified procedure of that by Steensgaard et al. [27] was used for the chlorosome isolation. Five grams of cell pellet was resuspended in 20 ml of the 50 mM Tris-HCl buffer containing 10 mM sodium ascorbate and 0.1% Triton X-100 (buffer A). After homogenization, the suspension was incubated with 1 mg DNase and 0.4 ml of 1 M $MgCl_2$ for 15 min in the dark at room temperature and then passed four times though an ice-cooled French pressure cell. After addition of 1.6 ml of 0.25 M EDTA (pH 7.0), the suspension of broken cells was incubated for 15 min in the dark at room temperature while being stirred. Unbroken cells and large debris were removed by centrifugation at $8000 \times g$. The supernatant was then applied on top of a step-wise sucrose density gradient (10, 20, 30, 40, 50, 60%, w/w) made with buffer A. After centrifugation at $150\,000 \times g$ for 18 h, chlorosomes and membrane-

rich fractions between 40 and 50% sucrose bands were recovered and the concentration of sucrose was adjusted to 60%. On top of this chlorosome and membrane-rich fraction, another step-wise sucrose density gradient was layered (0, 10, 20, 30, 40, 50% sucrose in the 50 mM Tris-HCl buffer containing 10 mM sodium ascorbate and 2 M NaSCN, w/w). After centrifugation at 150 000×*g* for 18 h, purified chlorosomes were recovered from the fraction between 10 and 20% sucrose. All centrifugations were performed at 4°C. Chlorosomes from the green sulfur bacterium *C. tepidum* ATCC 49652 were isolated according to the methods as previously described [5].

2.2. Alkaline treatment

Chlorosomes were treated with alkali as previously described [28,29] by adding 0.1 volume of 10 M NaOH to a chlorosome suspension in a 10 mM potassium phosphate buffer, pH 7.0 (final A_{742} was 5), and incubating the suspension at 40°C for 30 min (final pH was 12.7). After incubation, two volumes of a 1.0 M potassium phosphate buffer, pH 6.0, were added to obtain a final pH of 7.2 ± 0.2. The suspension was further diluted with the 10 mM potassium phosphate buffer and the chlorosomes were pelleted by centrifugation (300 000×*g* for 30 min at 4°C). The chlorosomes were washed twice with the 50 mM Tris-HCl buffer, resuspended in the same buffer and stored at −80°C.

2.3. Proteinase treatment

Chlorosomes were suspended in 400 μl of a 10 mM Tris-HCl buffer, pH 8.6 (final A_{742} was 50). After addition of 24 μl trypsin (1.5 mg/ml in water), the suspension was incubated at 37°C for 1 h in the dark. To terminate proteolysis, 15 μl of 200 mM phenylmethylsulfonyl fluoride in ethanol was added to the suspension and the chlorosomes were collected by centrifugation (300 000×*g* for 30 min at 4°C). The chlorosomes were washed twice with the 50 mM Tris-HCl buffer, resuspended in the same buffer and stored at −80°C until analysis.

Hexanol-detergent (HD)-treated chlorosomes obtained as described below were resuspended in the 10 mM Tris-HCl buffer and the BChl *a* concentration was adjusted to 10 μg/ml. Proteinase K was added to the chlorosome suspension so that the final concentration of proteinase K was 60 μg/ml. The suspension was then incubated at 55°C. After the incubation, proteins were precipitated by addition of 10–20-fold diethylether:ethanol (1:1), vacuum-dried and immediately analyzed by SDS-PAGE.

2.4. HD treatment

A hexanol-saturated buffer was prepared by adding approximately 0.01–0.02 volume of 1-hexanol to the 50 mM Tris-HCl buffer and shaking the mixture vigorously. To obtain a clear aqueous phase (H buffer) the mixture was left for at least 30 min at room temperature before use. Two hundred microliters of chlorosome stock (with an A_{742} of approximately 150) was injected in 6 ml of the H buffer and the suspension was vigorously shaken. The suspension was then diluted with an equal volume of solution D (distilled water containing 20% sodium cholate (w/v) and 0.1% Triton X-100 (v/v)). This mixture was incubated at 40°C for various periods in the dark. The chlorosomes were collected by ultracentrifugation, washed twice with the 50 mM Tris-HCl buffer, resuspended in a small volume of the same buffer and kept at −80°C until analysis.

2.5. Pigment quantification

Concentrations of BChl *a* and BChl *c* were determined spectroscopically by injecting a small volume of the sample into methanol. The absorption coefficients used were 68 mM^{-1} cm^{-1} at 770 nm for BChl *a* [30] and 70 mM^{-1} cm^{-1} at 669 nm for BChl *c* [31].

2.6. Absorption spectroscopy

Absorption spectra were recorded in a Shimadzu UV-160 spectrophotometer using a computer program specially written for that purpose.

2.7. SDS-PAGE

Chlorosome suspensions were mixed with the same volume of a sample buffer composed of 0.5 M Tris-HCl, pH 8.6, 20% glycerol (v/v), 8% SDS (w/v), 0.1% bromophenol blue (w/v) and 2% 2-mercaptoethanol

(v/v) and incubated for 2 min at 100°C. The heated samples were loaded onto gels containing 16.5, 10 and 4% acrylamide as separating, spacer and stacking gel, respectively, as described by Schägger and van Jagow [32]. After electrophoresis, the gels were stained with Coomassie brilliant blue R-250 (CBB) or with CBB and silver [33] using the Silver Stain 'DAIICHI' reagent purchased from Daiichi Pure Chemicals.

2.8. Protein analysis

CsmA protein from chlorosomes of *C. tepidum* and *C. aurantiacus* was recovered from the gel after SDS-PAGE by electrophoretically transferring it to polyvinylidene difluoride membranes. The N-terminal amino acid sequence of the recovered proteins was analyzed by means of an Applied Biosystems Model 477A sequencer with online PTH amino acid analysis (Model 121A).

For amino acid composition analysis, CsmA was isolated by an organic solvent extraction method [34]. A small volume of the chlorosome stock was injected into 50-fold of 80% acetone:diethylether:water (3:5:2, v/v) and CsmA was selectively extracted into the acetone-water phase. Half of the acetone-water phase was vacuum-dried, resuspended in 200 μl of 6 M HCl, hydrolyzed at 110°C for 22 h and analyzed by means of a Shimadzu amino acid analyzer (Model LC-6A). The remaining half of the acetone-water phase was used to determine the ratio of BChl *a* to CsmA. The phase was vacuum-dried, resuspended in the sample buffer and applied to SDS-PAGE together with SDS-solubilized proteins from various amounts of chlorosomes with known contents of BChl *a*. The amount of BChl *a* corresponding to CsmA in the isolate was estimated by densitometry of the gel after electrophoresis and comparing the band intensities of CsmA in the isolate to those in the SDS-solubilized chlorosomes.

3. Results

3.1. Alkaline and trypsin treatment

Chlorosomes isolated from *C. aurantiacus* were treated with strong alkaline conditions (pH 12.7 and 40°C for 30 min) or with proteinase trypsin (at 37°C for 1 h). Absorption spectra of untreated and treated chlorosomes are shown in Fig. 1. Untreated chlorosomes showed dominant peaks at 742 and 465 nm due to BChl *c* and a small band at 795 nm due to BChl *a*. The molar ratio of BChl *a* to BChl *c* in the untreated chlorosomes was approximately 0.07. The absorption properties of BChl *c* were not affected by alkaline treatment or trypsin treatment, whereas the 795 nm BChl *a* Q_y absorption band completely disappeared following alkaline treatment (Fig. 1). This is in good agreement with previous studies in which BChl *a* in chlorosomes isolated from *C. aurantiacus* and *C. tepidum* was chemically decomposed by alkaline treatment [28,29]. Trypsin treatment did not change the BChl *a* nor BChl *c* absorption properties.

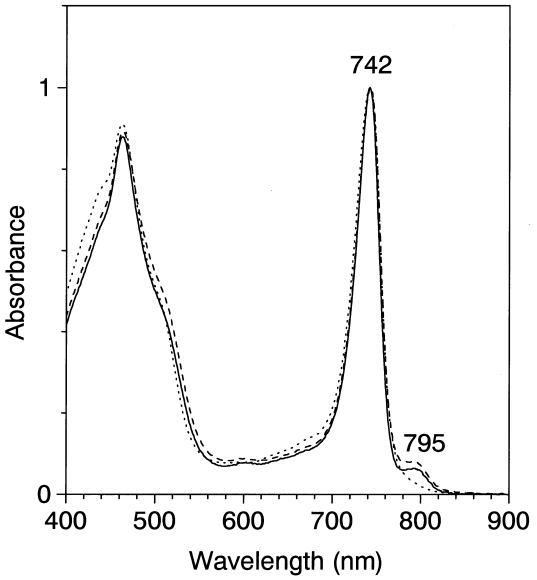

Fig. 1. Normalized absorption spectra of chlorosomes from *C. aurantiacus*. Solid line, untreated; dotted line, alkaline-treated and dashed line, trypsin-treated.

The effects of alkaline and trypsin treatment on chlorosomal proteins were investigated by SDS-PAGE. Six proteins were detected in untreated chlorosomes (Fig. 2A,B, lane 1). Three major proteins, CsmA, CsmN and CsmM, previously assigned with masses 3.7, 11 and 18 kDa [10,35], showed apparent masses of 3.8, 8 and 14 kDa in the current gel. Three minor proteins with apparent masses of 6, 9.5 and 21 kDa were also observed. The 6 kDa protein is probably identical to the 5.8 kDa protein previously reported by Feick and Fuller [10]. The CsmA, CsmN, CsmM and 6 kDa proteins could be visual-

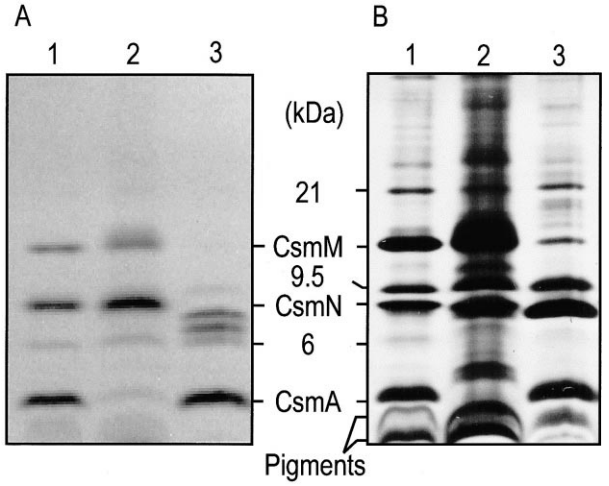

Fig. 2. Protein composition of (1) untreated chlorosomes, (2) alkaline-treated and (3) trypsin-treated chlorosomes from *C. aurantiacus* as determined by SDS-PAGE. A shows a gel stained by CBB and B shows a gel stained by CBB and silver. All samples were adjusted to contain 1 μg BChl *c*.

ized by CBB staining (Fig. 2A, lane 1). The 9.5 and 21 kDa proteins could be visualized only by silver staining (Fig. 2B, lane 1). Although CsmA was strongly stained by CBB, it was hardly stained by silver alone (data not shown). Alkaline treatment selectively removed CsmA (Fig. 2A,B, lane 2), while the other five proteins remained largely unaffected. A minor component appeared between CsmA and the 6 kDa protein (Fig. 2B, lane 2) may have originated from partial degradation of heavier proteins. When compared to the untreated sample, several protein bands appeared more intensive after the alkaline treatment, especially when the gel was stained with silver staining (Fig. 2B, lane 2). This may be due to a possible partial removal of BChl *c* by the treatment and due to degradation products of minor large proteins which are very sensitive to silver staining after the degradation. Trypsin treatment caused digestion of CsmN and CsmM, while the other four proteins were not changed (Fig. 2A,B, lane 3).

3.2. HD treatment

To confirm the correlation of CsmA to BChl *a* in chlorosomes, we developed a new method using hexanol and a detergent solution containing 20% sodium cholate and 0.1% Triton X-100 ('HD treatment'). As previously reported, BChl *c* aggregates in chlorosomes become reversibly 'monomerized' when the chlorosomes are suspended in a hexanol-saturated buffer and show a characteristic absorption peak at 668 nm due to monomeric BChl *c* [36,37]. When such a suspension of chlorosomes was diluted with the detergent solution mentioned above, the absorption peak at 668 nm was reduced significantly and a new broad peak appeared in the region of 730 nm. This represents a reversible conversion of the monomeric BChl *c* to an aggregated form. Fig. 3 shows the absorption spectra of HD-treated chlorosomes incubated for various periods and resuspended in the buffer after centrifugation. The BChl *c* absorption at 730 nm decreased relatively to the BChl *a* absorption at 795 nm during the incubation. This indicates that BChl *c* molecules were selectively extracted from the chlorosomes without affecting the absorption properties of BChl *a*. The extracted BChl *c* remained in solution in its monomeric form after centrifugation harvest of the chlorosomes (data not shown). During the incubation, the Q_y peak position of BChl *c* was slightly shifted to longer wavelengths, whereas that of BChl *a* did not change. In the region around 500 nm, two broad peaks became more visible within 12 h following the removal of BChl *c*. These peaks were attributed to carotenoids in the

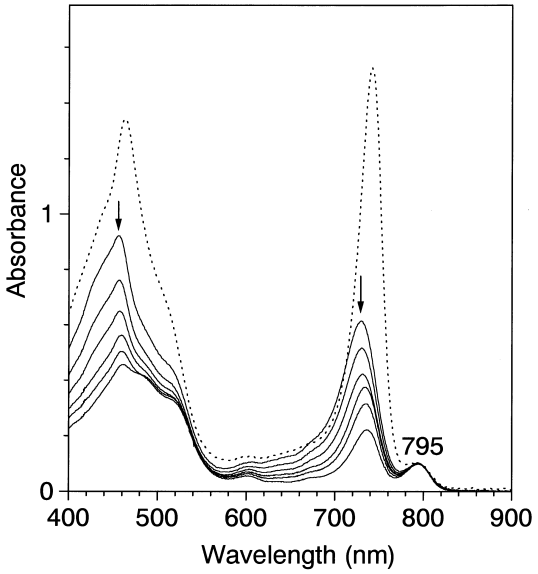

Fig. 3. Absorption spectra of untreated chlorosomes (dotted line) and HD-treated chlorosomes (solid lines) from *C. aurantiacus*. The chlorosomes were treated with hexanol and detergents (see Section 2) for 30 min, 1, 2, 4, 6, 12 h at 40°C. The arrows indicate the direction of the absorption changes. All the spectra have been normalized at 795 nm.

chlorosomes judged by their absorption wavelengths. Thus, carotenoids were apparently not extracted from the chlorosomes by the HD treatment. The peak around 600 nm is probably due to the Q_x peak of BChl *a*.

Fig. 4 shows changes in the molar ratio of BChl *c* to BChl *a* during the HD treatment of chlorosomes determined by absorption bands in methanol. This figure shows that the BChl *c* content decreased to 20% of the initial content after a 12 h HD treatment on the basis of BChl *a*.

The protein composition of the HD-treated chlorosomes is shown in Fig. 5. Among the four proteins detected by CBB staining, only the content of CsmA remained constant (Fig. 5A), whereas the intensity of the bands due to CsmM, CsmN and the 6 kDa protein was significantly reduced. The minor proteins (9.5 and 21 kDa) detected by silver staining also largely decreased following the HD treatment (Fig. 5B).

Chlorosomes, in which 80% of the BChl *c* was extracted by HD treatment, were further subjected to proteinase K treatment at 55°C. This proteolytic treatment caused a significant decrease in the BChl *a* absorption band at 795 nm accompanied by disappearance of the CsmA band in SDS-PAGE (data not shown).

3.3. Amino acid analysis of the CsmA protein

Højrup et al. have shown that a procedure involving precipitation with organic solvents preferentially extracted proteins smaller than 6 kDa from *Chlorobium* chlorosomes [34]. Applying the same procedure to *Chloroflexus* chlorosomes, we detected only CsmA in SDS-PAGE. The amino acid composition of the extracted protein was in good agreement with that from the deduced amino acid sequence of CsmA (data not shown). When a given volume of this CsmA extract was analyzed for amino acid compositions, it was found to contain approximately 90 pmol CsmA based on the contents of aliphatic and acidic amino acid residues (Ala, Gly, Ile, Leu, Pro, Val, Asx, Glx), which appeared to be most stable in our analytical procedure. The same volume of the extract was subjected to SDS-PAGE together with various volumes of chlorosomes with a known con-

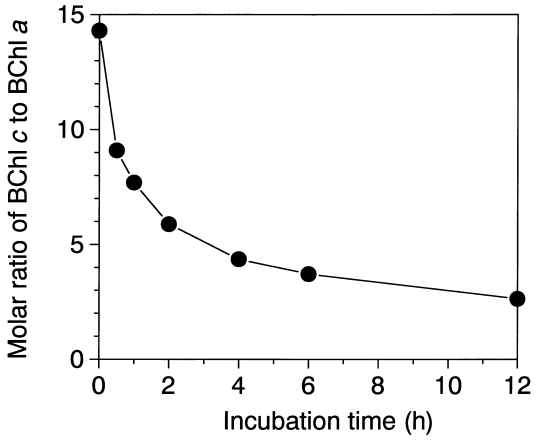

Fig. 4. The change of the molar ratio of BChls during HD treatment of chlorosomes. Each point corresponds to the spectra shown in Fig. 3.

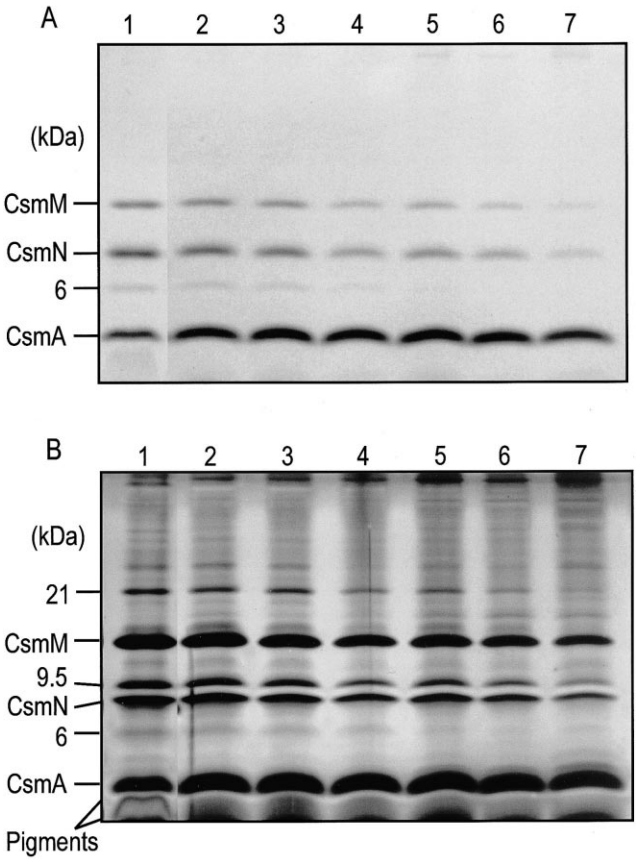

Fig. 5. Protein composition of HD-treated chlorosomes from *C. aurantiacus* as determined by SDS-PAGE. A shows a gel stained by CBB and B shows a gel stained by CBB and silver. For both panels: lane 1, untreated chlorosomes; lane 2, HD-treated chlorosomes for 30 min; lane 3, 1 h; lane 4, 2 h; lane 5, 4 h; lane 6, 6 h; lane 7, 12 h. The amount of chlorosomes loaded in each lane corresponds to 35 ng BChl *a*.

centration of BChl *a*. The relative content of CsmA in those samples was analyzed by densitometry and the BChl *a* content in the extract applied was estimated to be 110 pmol. Based on these results, the molar ratio of BChl *a* to CsmA was estimated to be approximately 1.2 mol per mol.

The molar ratio of BChl *a* to CsmA protein in isolated chlorosomes was also estimated by amino acid sequence analysis. The determined N-terminal nine residues in CsmA from *C. aurantiacus* were identical to those deduced from the gene sequence and the determined 13 residues in CsmA from *C. tepidum* were identical to those deduced from the gene sequence except for two unidentified residues (data not shown) [12,16]. Based on the detected amounts of the N-terminal amino acid residues, the molar ratios of BChl *a* to the CsmA protein were estimated to be 1.4–2.7 in *C. aurantiacus* and 0.6–1.2 in *C. tepidum*. These ratios were obtained by estimating a recovery efficiency in the electroblotting of between 50 and 100%, an efficiency of the coupling reaction between the N-terminal amino acid resides and phenyl isothiocyanate reagent of 50% and that one third of the PTH amino acids were injected into the high performance liquid chromatography system.

4. Discussion

Three treatments of isolated chlorosomes applied in this study led to the following observations about the CsmA protein and the organization of BChl *a* in chlorosomes from *C. aurantiacus*. Firstly, trypsin treatment showed that both CsmN and CsmM are unlikely to be involved in the BChl *a* protein complex absorbing at 795 nm since these two proteins were degraded, whereas the BChl *a* absorption was not changed. No other proteins were degraded by trypsin treatment. Secondly, the results of alkaline treatment suggested a correlation between CsmA and BChl *a* since only this protein was removed from the chlorosome along with reduction of the 795 nm absorption band. Thirdly, the HD treatment removed most BChl *c* molecules and all the detected proteins except CsmA from the chlorosome without affecting the 795 nm absorption band. Furthermore, when such BChl *c* and protein-depleted HD-treated chlorosomes were treated with proteinase K, both CsmA and the 795 nm absorption band were degraded. From these results, we concluded that CsmA is highly likely to be associated with BChl *a* in chlorosomes from *C. aurantiacus*.

Feick and Fuller [10] have proposed that a protein distinct from CsmA with an apparent molecular weight of 5.8 kDa may be associated with BChl *a* based on an observation that proteinase K digestion of this protein occurred in parallel with degradation of the 795 nm absorption band. However, in our observation on the HD treatment, this 5.8 kDa protein was extracted from the chlorosomes, whereas the 795 nm BChl *a* absorption band was not affected. Therefore, the 5.8 kDa protein is unlikely to be the BChl *a*-binding protein.

The localization of the chlorosomal proteins has been investigated on chlorosomes from *C. aurantiacus* by immuno gold labelling electron microscopy by Wullink et al. [13]. Their results suggested that CsmA is located in the chlorosome envelope, most pronounced in the side opposing cytoplasmic membranes. Considering the function of BChl *a* as the mediator of excitation energy from BChl *c* in chlorosomes to the reaction centers in the cytoplasmic membranes, BChl *a* in chlorosomes and its associated protein are expected to be located at the region in contact with the cytoplasmic membrane. One possible explanation for the apparent contradiction between our results and the results by electron microscopic observation is that the location of CsmA might not be restricted to the attachment site of the chlorosome to the cytoplasmic membrane, although this seems to be inconsistent with the current model of energy transfer [1]. However, since we do not yet have direct evidence about where BChl *a* is located in the chlorosomes, such an interpretation should not be ruled out. Another possible explanation is that the arrangement of the chlorosome attachment site was distorted during cell breakage when the chlorosome preparations were made. It has been reported that the efficiency of excitation energy transfer from BChl *c* in chlorosomes to the light-harvesting systems in the cytoplasmic membrane decreases to 45% when cells of *C. aurantiacus* are sonicated [37].

The molar ratio of BChl *a* to CsmA in chlorosomes from *C. aurantiacus* was estimated in this

study to be 1.4–2.7 by amino acid sequence analysis and 1.2 by amino acid composition analysis. These values are consistent with the above idea that CsmA is associated with BChl *a*, possibly in the ratio of one, two or three BChl *a* molecules per one CsmA molecule.

CsmA from green sulfur bacteria has a significant sequence homology of amino acids to that from *Chloroflexus*. Two regions with sequences of G-H-W or I-N-R/Q-N-A-Y are highly conserved [14,15]. The estimated molar ratio of BChl *a* to CsmA from *C. tepidum* was comparable to the ratio estimated in *C. aurantiacus*. Thus, we suggest that the CsmA protein is also involved in the BChl *a* organization in chlorosomes in green sulfur bacteria. Histidine is a very suitable candidate as a BChl ligand, as seen in the FMO protein [1] as well as in other photosynthetic apparatus such as reaction centers and light-harvesting systems in purple bacteria [38,39]. It may be also the case in chlorosomes that BChl *a* binds to CsmA using histidine as a ligand.

We have been trying to isolate BChl *a*-binding protein from chlorosomes for several years without success applying various methods to isolate membrane proteins. The monolayer nature of the chlorosome envelop may be a difficult factor to apply the normal procedure to isolate proteins from bilayer membranes. We are currently using a HD combination to try to isolate BChl *a* containing proteins without rod-forming BChl *c* and carotenoids.

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