ELSEVIER

FEBS Letters 342 (1994) 319–324

FEBS 13890

FEBS LETTERS

Structural differences in chlorosomes from *Chloroflexus aurantiacus* grown under different conditions support the BChl *c*-binding function of the 5.7 kDa polypeptide**

Rainer P. Lehmann, René A. Brunisholz, Herbert Zuber*

Institut für Molekularbiologie und Biophysik, ETH-Hönggerberg, 8093 Zürich, Switzerland

Received 19 January 1994; revised version received 7 March 1994

Abstract

Structurally different chlorosomes were isolated from the green photosynthetic bacterium *Chloroflexus aurantiacus* grown under different conditions. They were analysed with respect to variable pigment–protein stoichiometries in view of the presumed BChl *c*-binding function of the 5.7 kDa chlorosome polypeptide. Under high-light conditions on substrate-limited growth medium the pigment–protein ratio of isolated chlorosomes was several times lower than under low-light conditions on complex medium. Proteolytic degradation of the 5.7 kDa polypeptide in high-light chlorosomes led to a 60% decrease of the absorbance at 740 nm. The CD spectrum of high-light chlorosomes exhibited a sixfold lower relative intensity at 740 nm ($\Delta A/A_{740}$) than low-light chlorosomes, but it showed a fivefold increase in intensity upon degradation of the 5.7 kDa polypeptide compared to a twofold increase in low-light chlorosomes. It seems probable that BChl *c* in the chlorosomes is present as oligomers bound to the 5.7 kDa polypeptide. Our data suggest further that compared to low-light chlorosomes smaller oligomers or single BChl *c* molecules are bound to the 5.7 kDa polypeptide in high-light chlorosomes resulting in lower rotational strength.

Key words: Bacteriochlorophyll *c*; Circular dichroism; Green photosynthetic bacterium; Light-harvesting antenna; Pigment–protein interaction

1. Introduction

The light-harvesting system of the green photosynthetic bacterium *Chloroflexus aurantiacus* is composed of the chlorosomes, which contain BChl *c* and BChl *a* as light-harvesting pigments, and the B806–866 BChl *a*–protein complex, which is integrated within the CM surrounding the reaction centre [1]. The chlorosomes are ellipsoid bodies of an average size of $110 \times 30 \times 12$ nm^3 attached to the inner side of the CM [2].

Controversy exists regarding the organization of BChl *c* in the chlorosomes. Evidence based on biochemical experiments suggested that the BChl *c* molecules are bound in BChl *c*–protein complexes [3,4]. Three major polypeptides were found to be present in all chlorosome preparations [5], namely the 18 and the 11 kDa polypeptide and the 5.7 kDa polypeptide, which was thought to bind the BChl *c* molecules [6]. Spectroscopic findings on the other hand indicated that BChl *c* in chlorosomes could be organized as oligomers, where pigment–pigment interactions are responsible for the observed red shift of the light-harvesting pigments [7–9].

Under certain growth conditions *Chloroflexus aurantiacus* cells increase the amount of BChl *c* and seem to incorporate newly synthesized BChl *c* into preformed chlorosomes [10]. It was demonstrated that chlorosomes can increase in volume when cells produce more BChl *c* and the idea of a variable degree of packing of BChl *c* in chlorosomes was put forward [11]. Interestingly, an increase in BChl *c* in the chlorosomes is not accompanied by a proportional increase of the chlorosome polypeptides indicating variable pigment–protein stoichiometries [12].

In order to explain the variable pigment–protein stoichiometries observed in chlorosomes and to add strength to the previous proposal of the pigment-binding function of the 5.7 kDa polypeptide we used different growth conditions for the cultivation of *Chloroflexus aurantiacus* and obtained structurally different chlorosomes. We have compared the different chlorosomes with respect to their absorption and circular dichroistic properties as well as their response to proteolytic treatment.

* Corresponding author.

** Dedicated to Prof. Dr. Dres. h.c. A. Trebst on the occasion of his 65th birthday.

Abbreviations: BChl, bacteriochlorophyll; CD, circular dichroism; CM, cytoplasmic membrane.

2. Materials and methods

2.1. Growth conditions

Chloroflexus aurantiacus J-10-fl was grown photoheterotrophically in 1 litre batches at 57°C under two different growth conditions desig-

SSDI 0014-5793(94)00275-Z

nated as low-light and high-light conditions. For low-light conditions incandescent light of about 150 $W \cdot m^{-2}$ was used. The growth medium contained per litre: 100 mg ethylenediaminetetraacetic acid, 60 mg $CaSO_4 \times 2H_2O$, 100 mg $MgSO_4 \times 7H_2O$, 8 mg NaCl, 110 mg Na_2HPO_4, 130 mg KNO_3, trace elements, the vitamin mixture described in [13], 1.0 g yeast extract, 0.5 g glycyl-glycine, 2.5 g casamino acids. The cells were grown for 72 h. For high-light conditions tungsten spot lights of about 2000 $W \cdot m^{-2}$ were used. The growth medium contained the above salts apart from 130 mg KNO_3, which was replaced by 130 mg potassium acetate; trace elements, 100 mg biotin, 0.25 g yeast extract, 0.5 g glycyl-glycine, 0.3 g glutamic acid. The cells were grown for 22 h to 48 h depending on the experiment.

2.2. Chlorosome isolation

Chloroflexus aurantiacus cells were washed with 10 mM Tris-HCl, pH 8.0 and disrupted by ultrasonication. Whole cells and large cell debris were sedimented by centrifugation at 27,000 × *g* for 15 min. The membrane with the chlorosomes (whole-membrane fraction) was prepared by centrifugation at 100,000 × *g* for 1 h. The whole-membrane fraction was resuspended in buffer with a Potter-Elvehjem homogenizer and adjusted to an absorbance of 10 at 865 nm. For solubilization 5% w/v Miranol S2M-SF was added and the suspension was stirred at room temperature in the dark for 30 min. The solubilized chlorosomes were separated on a sucrose density gradient and subsequently washed two times with 10 mM Tris-HCl, pH 8.0 by centrifugation at 100,000 × *g*.

2.3. Pigment determination

BChl *c* and BChl *a* were quantitatively determined as described in [5].

2.4. Proteolytic treatment

A suspension of chlorosomes in buffer was adjusted to an absorbance of 2 at 740 nm. 1 μg of proteinase K or trypsin per 1 ml of chlorosome suspension was added (in 45 min intervals) for proteolytic treatment at 30°C for 2 h. The digestion was stopped by adding a serine protease inhibitor (5 mM Pefabloc SC, Pentapharm AG, Basel, Switzerland) and the absorption and CD spectra were recorded immediately afterwards. Aliquots for analysis on SDS-PAGE were taken out of the samples and denatured immediately after the spectral measurements.

2.5. Spectroscopy

Absorption spectra from 250 nm to 900 nm were recorded on a Perkin Elmer Lambda 5 UV/VIS spectrophotometer with a 1 cm quartz cuvette at room temperature. The slit width was 1 nm and the scan speed 60 nm/min. The response time was set to 0.5 s. Circular dichroism spectra from 300 nm to 800 nm were recorded on a Jasco J-710 spectropolarimeter with a cuvette of 1 mm pathlength at 22°C. The slit width was 2 nm and the scan speed 20 nm/min. The response time was set to 2 s. The spectra were accumulated three times and smoothed using the noise reduction option of the J-700 system software.

2.6. Electrophoresis

Polyacrylamide gel electrophoresis was performed using the Mini-Protean II dual slab cell system (Bio-Rad Laboratories) according to [14] with a total acrylamide content of 16.5% and 3% of crosslinking reagent in the separating gel. The electrophoresis buffer contained 0.1 M Tris base, 0.1 M Tricine (*N*-tris[hydroxymethyl]-methylglycine) and 0.1% SDS. Chlorosome samples were precipitated with 10% trichloroacetic acid and extracted two times with cold acetone. The samples were dried and dissolved in buffer composed of 62.5 mM Tris-HCl, pH 6.8, 10% glycerol, 4% SDS, 0.05% Bromophenol blue, 5% 2-mercaptoethanol and incubated for 2 min at 100°C.

3. Results

Chloroflexus aurantiacus was grown under low-light and high-light conditions. The high-light cultures were additionally grown for different periods of time. The effect of low-light and high light conditions on the pigment composition of the whole-membrane fraction and the isolated chlorosomes as well as the influence of the length of growth on the high-light cultures can be seen in the absorption spectra (Fig. 1A,B) and is summarized in Table 1. Under low-light conditions the ratios of BChl *c* to BChl *a* of the whole-membrane fraction and of the isolated chlorosomes were ten times higher compared to the respective fractions from cells grown under high-light conditions for 22 h. Prolonged growth under high-light conditions led to an increase of the BChl *c* to BChl *a* ratios. Based on the absorption intensity at 268 nm the absorbance at 740 nm was about 5 times higher in low-light chlorosomes. Analysis on SDS-PAGE revealed that, based on the same amounts of BChl *c*, the high-light chlorosomes contained several times more of the 3 major chlorosome polypeptides (11, 18 and 5.7 kDa) and additional bands in the higher molecular weight range (Fig. 2).

The CD spectra of chlorosomes isolated from cells grown under low light and high-light conditions showed different intensities of the near infra-red signal. A typical CD spectrum for low-light chlorosomes as shown in Fig. 3A had a strong, S-shaped signal centred at 740 nm. Based on the absorbance of the 740 nm band ($\Delta A/A_{740nm}$) the high-light chlorosomes exhibited a sixfold weaker CD effect in the 740 nm region (Fig. 3A) than low-light chlorosomes. The relative intensities of the CD signals at 740 nm of chlorosomes isolated from high-light cultures increased by a factor of two upon prolonged growth. The near infra-red CD signals of the different chlorosome preparations were about the same with respect to the position of the zero-crossing (740 nm), the positive maximum (755 nm) and the negative maximum (725 nm). Hence, it seems unlikely that different growth

Table 1
Effect of light intensity and duration of growth on the pigment composition of the whole-membrane fraction and the isolated chlorosomes

Growth conditions	Whole-membrane fraction		Isolated chlorosomes		
	A_{740}/A_{866}	BChl *c*/BChl *a* (mol/mol)	A_{740}/A_{796}	A_{740}/A_{268}	BChl *c*/BChl *a* (mol/mol)
Low-light	25	11	15	3.4	18
High-light 22 h	2.5	0.9	2.3	0.65	1.8
High-light 30 h	3.3	1.4	4.5	1.2	3.0
High-light 48 h	3.4	1.6	5.7	1.4	3.7

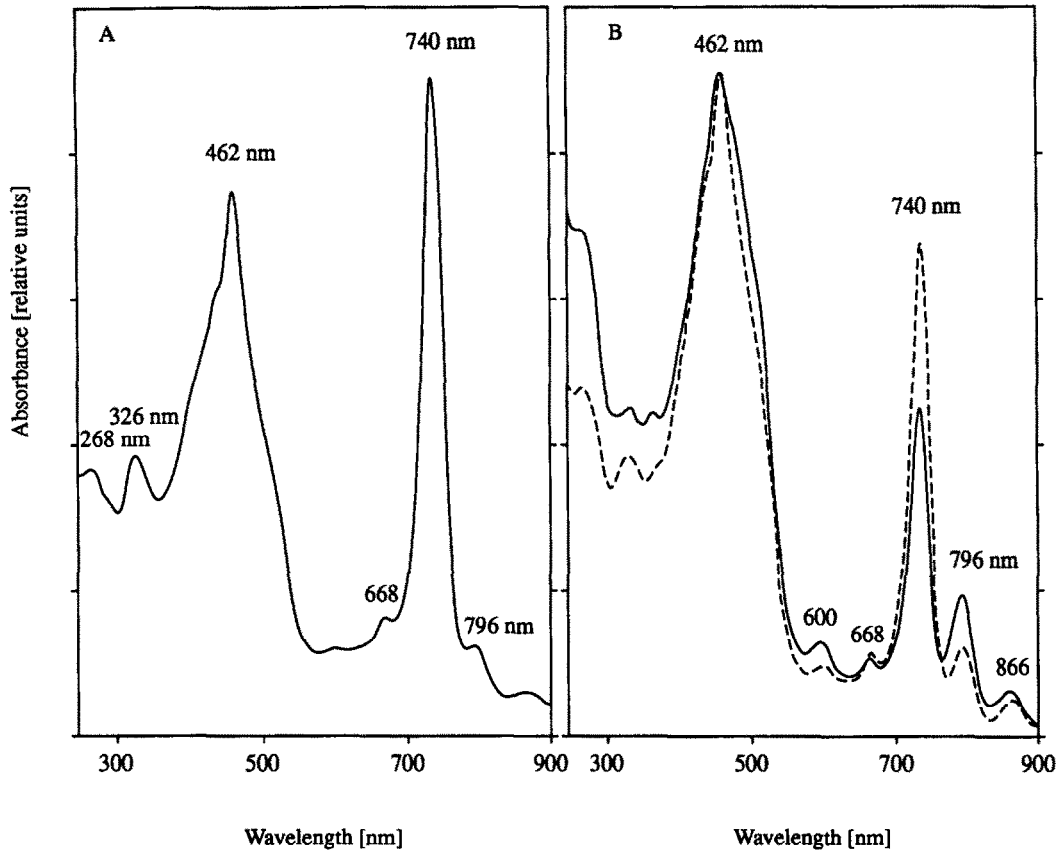

Fig. 1. Absorption spectra of isolated chlorosomes in 10 mM Tris-HCl, pH 8.0. (A) Chlorosomes isolated from low-light grown *Chloroflexus aurantiacus*. (B) Solid line, isolated from 22 h high-light grown cells; dashed line, isolated from 48 h high-light grown cells.

conditions were the origin of the reversed signals published earlier [15–17].

Low light and high-light chlorosomes were affected to different extents by proteolytic treatment. As can be seen in Table 2 low light chlorosomes hardly responded to proteolytic treatment. The degradation of the 11 and 18 kDa polypeptides with trypsin did not change the absorption intensity of the 740 nm band at all. Treatment with proteinase K, which partly degraded the 5.7 kDa polypeptide in addition to the 11 and 18 kDa polypeptide, led only to a very slight decrease of the 740 nm band. In high-light chlorosomes, however, trypsin brought about a 9% decrease of the absorption intensity at 740 nm and proteinase K caused a 60% decrease (see Fig. 4 for the time course). At the same time a 3 nm blue shift of the 740 nm band could be observed and the 668 nm absorption band of liberated BChl *c* came up in the spectrum. Analysis on SDS-PAGE revealed that the 5.7 kDa polypeptide was completely degraded by proteinase K in high-light chlorosomes (not shown).

The relative CD intensity ($\Delta A/A_{740nm}$) of the low-light chlorosomes doubled upon digestion with proteinase K but it did not change after treatment with trypsin [18]. In the case of the 48 h high light chlorosomes the relative intensity increased fivefold after the degradation of the 5.7 kDa polypeptide with proteinase K and remained constant with trypsin (Fig. 3B).

4. Discussion

The composition of the photosynthetic apparatus of *Chloroflexus aurantiacus*, especially the BChl *c* to BChl *a* ratio, depends on the growth rate [19] and on other

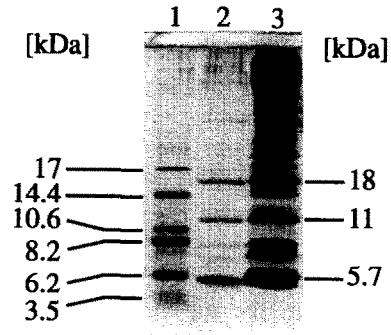

Fig. 2. SDS-PAGE showing the polypeptide patterns and the difference in the pigment–protein stoichiometries of low-light and high-light chlorosomes. Lane 1, low molecular weight standard (Sigma); lane 2, low-light chlorosomes; lane 3, high-light chlorosomes. Both chlorosome samples contained the same amount of BChl *c*.

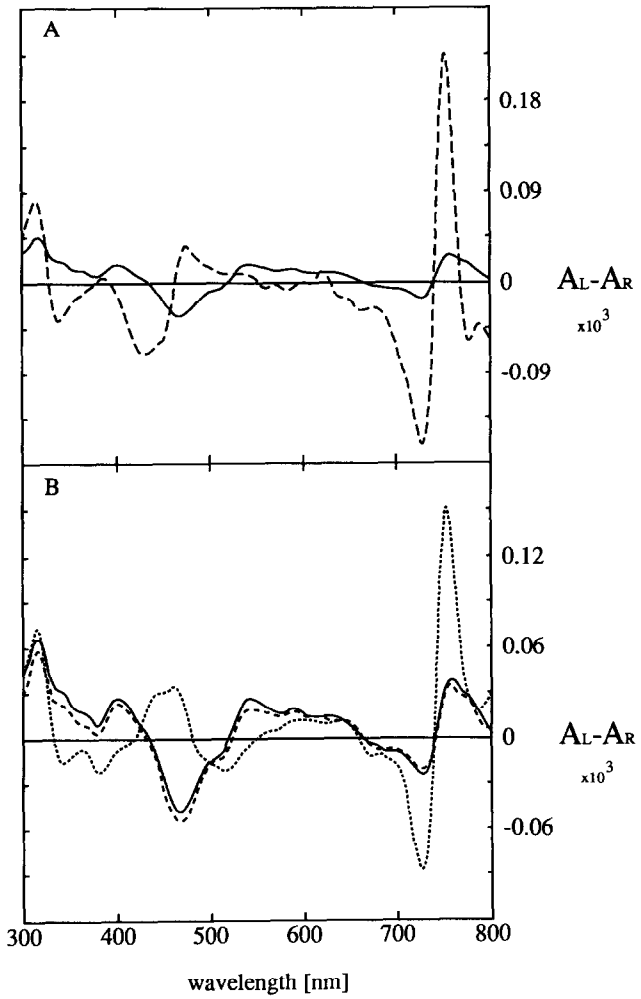

Fig. 3. (A) CD spectra of native chlorosomes adjusted to an absorbance of 2.7 at 740 nm: dashed line, low-light chlorosomes; solid line, 48 h high-light chlorosomes. (B) CD spectra showing the proteolytic treatment of 48 h high-light chlorosomes: solid line, 48 h high-light chlorosomes with an absorbance of 3.7 at 740 nm; dashed line, sample treated with trypsin for 2 h at 30°C; dotted line, sample treated with proteinase K for 2 h at 30°C. The pathlength of the cuvette was 1 mm.

factors such as light intensity [20], oxygen [21] and nutritional factors [22]. As we used glutamate as the major carbon source for high-light grown cells, it is possible that BChl *c* synthesis was impaired because protein and BChl synthesis competed for the same substrate [21,23]. Therefore, in our experiments, it is not possible to decide whether nutritional or light induced effects (indirectly the growth rate) contributed to the structural differences observed in the different chlorosome preparations.

The structural differences of chlorosomes isolated from cells grown under low-light and high-light conditions are reflected in the different stoichiometries between pigments and proteins. Low light chlorosomes have a molar ratio of BChl *c* molecules to the 5.7 kDa polypeptide of 15–21 : 1 ([24]; Lehmann, unpublished data). The exact value for high-light chlorosomes could not be determined, but judging from the staining intensities on SDS-PAGE the ratio is about five times lower for the 22 h high-light chlorosomes compared to the low-light sample. The absorbance ratio of A_{740nm} to A_{268nm} (there was no absorbance maximum at 280 nm) also differed by a factor of five between the low and the 22 h high-light chlorosomes.

Secondly, the pronounced differences in the susceptibility of the 740 nm absorption band to proteases is apparently due to the fact that in low-light chlorosomes the 5.7 kDa polypeptide is much less accessible to proteases. Supporting these findings, proteolytic treatment using thermolysin [4] showed that low-light chlorosomes were not affected by the enzyme below 40°C and equally, thermolysin treatment produced a stronger decrease of the 740 nm band in medium light chlorosomes.

Thirdly, there must be a considerable difference in the aggregation state of BChl *c* between the low-light and the high-light chlorosomes, which is reflected in the different relative intensities of either CD signals. We observed that the CD intensity of low-light chlorosomes only doubled upon treatment with proteinase K compared to a fivefold increase in the high-light chlorosomes. There is some evidence that BChl *c* in the chlorosome is organized as oligomers [8,9], which may be bound to protein in order to maintain parallel transition moments between clusters of BChl *c* [25,26]. There are several reports that BChl *c* aggregates in hexane [27,28], in aqueous suspension, or after LDS treatment [4], and after proteolytic treatment [4,18] exhibited higher rotational strength than BChl *c* in chlorosomes of *Chloroflexus aurantiacus*. Similarly, GEF-chlorosomes [29] showed stronger CD bands than chlorosomes isolated with detergents and density gradient centrifugation, according to a comparative study [28]. In an aqueous aggregate of a chloroform/methanol extract from *Chloroflexus aurantiacus* chlorosomes, however, the rotational strength was lower than in the chlorosomes [30]. Moreover, chlorosomes from *Chlorobium limicola* were reported to have a stronger CD signal than oligomers in methylene chloride/hexane (1 : 200) [7].

We conclude that, if the intensity of the CD signal around 740 nm in the chlorosomes of *Chloroflexus aurantiacus* is depending on the aggregate size of BChl *c*

Table 2
Remaining absorption intensity and blue shift of the 740 nm absorption band after proteolytic treatment with trypsin or proteinase K at 30°C for 2 h

	Low-light chlorosomes		High-light chlorosomes[a]	
	Remaining intensity (%)	Blue shift (nm)	Remaining intensity (%)	Blue shift (nm)
Control	97	0	92	0
Trypsin	97	0	84	1
Proteinase K	95	0	37	3

[a] Chlorosomes isolated from cells after 22 h growth under high-light conditions.

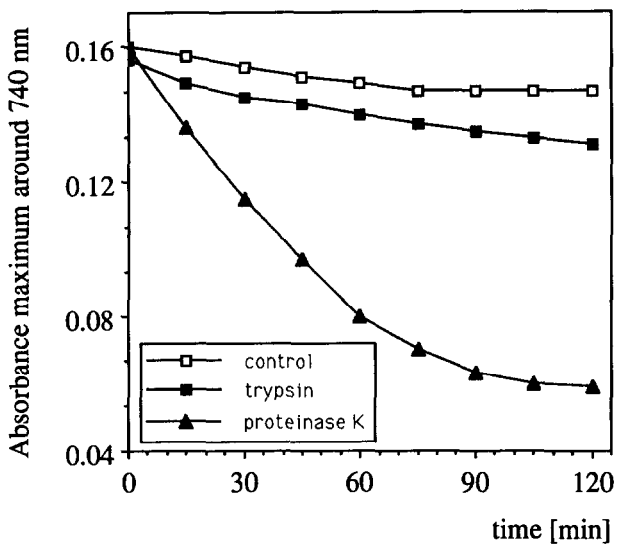

Fig. 4. Time course of the proteolytic treatment of 22 h high-light chlorosomes at 30°C using trypsin and proteinase K. Enzyme was added in 45 min intervals.

[4,18], the BChl *c* oligomers in high-light chlorosomes are smaller than in low-light chlorosomes. In that sense, the degradation of the 5.7 kDa polypeptide in high-light chlorosomes would produce a stronger increase in rotational strength, because only then the liberated pigments form larger oligomers. Therefore, we assume that the 5.7 kDa polypeptide is associated with BChl *c* in the chlorosomes. We propose that a certain number of BChl*c* molecules may be bound to the 5.7 kDa polypeptide, e.g. to the imidazole nitrogen of one histidine, which is also a conserved residue in the sequences of the 6.3 kDa polypeptides of the chlorosomes from Chlorobiaceae [31], and possibly to the carbonyl oxygens of the amide groups of glutamine and asparagine residues [6]. Additional BChl *c* molecules could be bound as oligomers to the polypeptide-bound BChl *c* molecules, which would result in higher pigment–protein ratios. In this way, the chlorosomes could respond to different growth conditions by increasing the number of pigments per polypeptide.

The energy collected by the BChl *c*-antenna (B740) is further transferred onto a BChl *a* based antenna complex (B790) within the chlorosome, the so-called base-plate. In contrast to our observations, a constant ratio of BChl *c* to BChl *a* of 25 : 1, which was independent of growth conditions, was reported earlier [5]. BChl *a* as part of the chlorosomes is thought to be associated with a polypeptide in the pigment–protein complex of the base-plate [3]. The polypeptide pattern of high-light chlorosomes with a strong B790 absorption band, i.e. a low BChl *c* to BChl *a* ratio, comprises the three major chlorosome proteins (18, 11 and 5.7 kDa), but there is no valuable candidate for the base-plate protein in the 6–8 kDa range (Fig. 2). The two bands which are located between the 11 and the 5.7 kDa band were identified as the B806–866-β (lower band) and an unknown amino acid sequence (upper band). The N-terminus of the unknown sequence was determined, namely SVKESGGIFGMMFREVGFFW-EQV-NWAEE, and it remains unclear, whether this represents the base-plate protein or whether the base-plate protein is located among the higher molecular weights, which were not resolved in our SDS-PAGE system.

Acknowledgements: We are grateful to Prof. J. Oelze, Universität Freiburg (Germany), for his helpful advice concerning the cultivation of *Chloroflexus aurantiacus*. We wish to thank Dr. G. Frank (ETH Zürich) for the protein sequencing and Ms M. Rümbeli-Wirth as well as Ms V. Eggli (ETH Zürich) for excellent technical assistance. This work was supported by the ETH Kredit Unterricht und Forschung and the Schweizerischer Nationalfonds (Project Nos. 31–25179.88; 31–32071.91).

References

[1] Zuber, H. and Brunisholz, R.A. (1991) in: Chlorophylls (Scheer, H., Ed.) pp. 627–703, CRC Press, Boca Raton, FL.
[2] Staehelin, L.A., Golecki, J.R., Fuller, R.C. and Drews, G. (1978) Arch. Microbiol. 119, 269–277.
[3] Feick, R.G. and Fuller, R.C. (1984) Biochemistry 23, 3693–3700.
[4] Niedermeier, G., Scheer, H. and Feick, R.G. (1992) Eur. J. Biochem. 204, 685–692.
[5] Feick, R.G., Fitzpatrick, M. and Fuller, R.C. (1982) J. Bacteriol. 150(2), 905–915.
[6] Wechsler, T., Suter, F., Fuller, R.C. and Zuber, H. (1985) FEBS Lett. 181(1), 173–178.
[7] Olson, J.M., Gerola, P.D., VanBrakel, G.H., Meiburg, R.F. and Vasmel, H. (1985) in: Antennas and Reaction Centers of Photosynthetic Bacteria (Michel-Beyerle, M.E., Ed.) pp. 67–73, Springer, Berlin.
[8] Brune, D.C., Nozawa, T. and Blankenship, R.E. (1987) Biochemistry 26, 8644–8652.
[9] Fetisova, Z.G. and Mauring, K. (1992) FEBS Lett. 307(3), 371–374.
[10] Schmidt, K., Maarzahl, M. and Mayer, F. (1980) Arch. Microbiol. 127, 87–97.
[11] Golecki, J.R. and Oelze, J. (1987) Arch. Microbiol. 148, 236–241.
[12] Oelze, J., Foidl, M. and Golecki, J.R. (1992) in: Research in Photosynthesis (Murata, N., Ed.) pp. 89–92, Kluwer Academic Publishers, The Netherlands.
[13] Madigan, M.T., Petersen, S.R. and Brock, T. (1974) Arch. Microbiol. 100, 97–103.
[14] Schägger, H. and VonJagow, G. (1987) Anal. Biochem. 166, 368–379.
[15] Betti, J.A., Blankenship, R.E., Natarajan, L.V., Dickinson, L.C. and Fuller, R.C. (1982) Biochim. Biophys. Acta 680, 194–201.
[16] VanDorssen, R.J., Vasmel, H. and Amesz, J. (1986) Photosynth. Res. 9, 33–45.
[17] Brune, D.C., Gerola, P.D., and Olson, J.M. (1990) Photosynth. Res. 24(3), 253–264.
[18] Lehmann, R.P., Brunisholz, R.A. and Zuber, H. (1994) Photosynth. Res., in press.
[19] Oelze, J. and Fuller, R.C. (1987) Arch. Microbiol. 148, 132–136.
[20] Pierson, B.K. and Castenholz, R.W. (1974) Arch. Microbiol. 100, 283–305.
[21] Oelze, J. (1992) J. Bacteriol. 174(15), 5021–5026.
[22] Oelze, J. and Söntgerath, B. (1992) Arch. Microbiol. 157, 141–147.
[23] Oelze, J., Jürgens, U.J. and Ventura, S. (1991) Arch. Microbiol. 156, 266–269.

[24] Eckhardt, A., Brunisholz, R., Frank, G. and Zuber, H. (1990) FEBS Lett. 267(2), 199–202.
[25] VanAmerongen, H., Vasmel, H. and VanGrondelle, R. (1988) Biophys. J. 54, 65–67.
[26] Fetisova, Z.G., Freiberg, A.M. and Timpmann, K.E. (1988) Nature 334, 633–634.
[27] Blankenship, R.E., Brune, D.C., Freeman, J.M., King, G.H., McManus, J.D., Nozawa, T., Trost, T. and Wittmershaus, B.P. (1988) in: Green Photosynthetic Bacteria. (Olson, J.M., Ormerod, J,G., Amesz, J., Stackebrandt, E. and Tüper, H.G., Eds.) pp. 57–68, Plenum Press, New York.
[28] Griebenow, K., Holzwarth, A. R., VanMourik, F. and VanGrondelle, R. (1991) Biochim. Biophys. Acta 1058, 194–202.
[29] Griebenow, K. and Holzwarth, A.R. (1989) Biochim. Biophys. Acta 973, 235–240.
[30] Miller, M., Gillbro, T. and Olson, J.M. (1993) Photochem. Photobiol, 57(1), 98–102.
[31] Wagner-Huber, R., Fischer, U., Brunisholz, R., Rümbeli, M., Frank, G. and Zuber, H. (1990) Z. Naturforsch. 45c, 818–822.