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Development and Pigmentation of Chlorosomes in *Chloroflexus aurantiacus* Strain Ok-70-fl

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Abstract. The development of chlorosomes and their pigmentation were studied by growing *Chloroflexus aurantiacus* strain Ok-7o-fl first under conditions under which BChl *c*-synthesis is low (50° C, 2000 lux and 39° C, 1500 lux) and subsequently under conditions promoting high BChl *c*-synthesis (50° C, 400 lux). Electron microscopic observations on and chemical analyses of isolated cell components showed that in BChl *c*-depleted cells chlorosome-like structures ("chlorosome bags") are attached to fragments of cytoplasmic membranes. These chlorosome bags exhibit a periodic fine structure caused by the construction of the baseplates of the chlorosomes. The baseplates are closely attached to the cytoplasmic membrane, they are rich in phospholipids and apparently contain a 790 nm-BChl *a*-complex. Chlorosome bags of BChl *c*-depleted cells always contain a limited amount of ligth-harvesting pigment complexes (BChl *c*, γ- and β-carotene). The light-harvesting system is restored (50° C, 400 lux) by first refilling the existing chlorosome bags before cell division takes place.

Key words: *Chloroflexus aurantiacus* – Chlorosomes – Cytoplasmic membranes – Bacteriochlorophyll – Carotenoids – Pigment-protein complexes.

Cells of *Chloroflexus aurantiacus* very sensitively respond to alterations in growth conditions with changes in pigmentation. This change concerns mainly the BChl *c*:BChl *a* ratio (Pierson and Castenholz, 1974, 1978). From the analytical data it was concluded that the differences in BChl-content of cells, which were grown at different light intensities, were mainly due to the amount of BChl *c* present. The BChl *a*-content was only slightly influenced. Since the bulk of BChl *c* (the light-harvesting pigment) is housed in the chlorosomes BChl *c*-synthesis was assumed to be controlled by the number of chlorosomes per cell.

Recently a study on the influence of light intensities on the chlorosomes and their pigmentation of a *Chlorobium limicola* strain was published by Broch-Due et al. (1978). The data demonstrated that low light-cells (22 lux) with high BChl *d*-content had enlarged chlorosomes. When cells were grown at 22,000 lux they contained only half the amount of BChl *d* as compared to the 22 lux-cells, and the chlorosomes appeared to be considerably smaller in size. Whether the number of chlorosomes was also affected could not be shown. The BChl *a*-content remained unchanged under both light conditions.

Holt et al. (1966) demonstrated a fourfold increase in BChl *c*-content in "*Chloropseudomonas ethylica*" when growing the cells at 107,600 and 100 lux, respectively.

The experiments of Pierson and Castenholz (1974) indicated that *Chloroflexus* responds much more pronounced to changes of light intensities than *Chlorobium* strains do. A hundredfold decrease in light intensity causes an about fourteenfold increase in BChl *c*-content. In contrast, at light intensities higher than 55,000 lux the BChl *c*-level is lower than that of BChl *a*. It was observed in our laboratory that high light-cells with low BChl *c*-content very quickly regenerate their light-harvesting system after being transferred to low light (400 lux) conditions. These observations suggested another kind of regulation for BChl *c*-synthesis functioning in *Chloroflexus* than was claimed for Chlorobiaceae. The possible existence of alternate regulatory mechanisms for pigment synthesis in Chlorobiaceae and Chloroflexaceae was briefly discussed by Pierson and Castenholz (1978). But so far, no

Abbreviations: BChl = Bacteriochlorophyll; LH = Light-harvesting complex; RC = Reaction center

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experimental evidence has been presented to support these ideas. This study, therefore, aimed at obtaining more detailed information about the development of chlorosomes and the regulation of pigmentation by characterizing isolated membrane fractions from *Chloroflexus* cells which were grown under conditions of restricted BChl *c*-synthesis and regeneration of the restricted light-harvesting pigment system.

Materials and Methods

Organism and Growth Conditions. Chloroflexus aurantiacus strain Ok-7o-fl (DSM 636) was used throughout this work. Cells were grown in 250 ml-screw capped flasks in a modified medium of Pierson and Castenholz (1974) as described by Schmidt (1980). The following growth contitions were chosen: a) 50° C, 400 lux, 96 h (low light-cells), b) 50° C, 2,000 lux, 48 h (high light-cells), c) 38 – 39° C, 1,500 lux, 8 days (low temperature-cells), d) as c) but then transferred to 50° C, 400 lux for 24 h or e) 48 h, without changing the medium.

Isolation of Cell Fragments and Chemical Analyses. Cell fragments (chlorosomes and cytoplasmic membranes and the corresponding fractions) were isolated from broken cells by repeated sucrose gradient centrifugation as described in a previous paper (Schmidt, 1980). The isolated fractions were analysed for their absorption spectra, pigmentation, contents of phospholipids, glycolipids, cytochrome *c*, and assayed for their activity of succinate dehydrogenase. Methods used for these analyses were the same as described by Schmidt (1980).

SDS-Polyacrylamide Gel Electrophoresis. SDS-acrylamide electrophoresis was carried out by the method of Weber et al. (1972) on 10% polyacrylamide gels. Proteins of all fractions were solubilized by boiling the samples in 0.01 M phosphate buffer (pH 7.0), containing 1% SDS and 1% 2-mercaptoethanol, for 2 min. The electrophoresis was run at room temperature with 2 mA per gel for the first hour and with 6 – 7 mA per gel until the ion front had passed about 7 cm of the gel. Cytochrome *c*, chymotrypsinogen, aldolase, and catalase were used as standard proteins.

Electron Microscopy. Negative staining was done according to Valentine et al. (1968) with 4% (w/v) uranyl acetate. Electron micrographs were taken with a Philips EM 301.

Results

The experiments of Pierson and Castenholz (1974) and the data of those to be reported in this paper revealed that a decrease in light intensity from 2,000 lux to 400 lux caused about a fourfold increase in BChl *c*-content in both *Chloroflexus* strains, J-10-fl (P. and C.) and Ok-70-fl (S. et al.) at 45° C and 50° C, respectively. This phenomenon could clearly be recognized by the colour change of the suspension: cells grown at 400 lux exhibited an intense green colour, those grown at 2,000 lux looked orange-yellow with a faint greenish shine. The filaments of 2,000 lux-suspensions tended to settle at the bottom of the flasks to form small colonies. These colonies developed a green colour as soon as they started to form denser cell concentrations because of self-shading. Therefore, at 2,000 lux flasks were shaken every few hours during incubation and the cells were harvested after 48 h of growth, before the suspension reached to high a density.

Growth and pigmentation of *Chloroflexus* was considerably, although not completely, inhibited by incubating the cells at 37 – 39° C. Below 37° C no growth was observed. Low temperature-cells for the analyses described in this paper were grown at 38 – 39° C. It was necessary to irradiate the flasks at light intensities of, at least, 1,000 or 1,500 lux to achieve significant growth. The incubation lasted about 8 days. After this time a relatively thin suspension of a pale or orange-yellow colour was obtained with a very little greenish gleam of BChl.

In order to promote BChl-synthesis in BChl-depleted cells grown at low temperature the flasks were transferred to 50° C and 400 lux for either 24 or 48 h. It could be recognized from the increase in greenish colour after 20 – 24 h that BChl-synthesis had started soon after the change of temperature and light intensity. Analyses of whole cells revealed that after 24 h the amount of BChl had doubled, although there was no measurable increase in protein content. This increase was almost exclusively due to BChl *c*-synthesis. After 48 h the suspensions were fairly green. In general the protein content was twice as high as in the original low temperature-cells but the BChl-content was about tenfold higher, again mainly caused by BChl *c*-synthesis. In this case the major part of the BChl *c*-increase appeared to be related to cell division and the formation of a population in which most of the cells are furnished with a set of chlorosomes typical of low light-cells.

Isolation and Characterization of Cell Fragments

Because of the sparse suspensions of cells grown at 50° C, 2,000 lux for 48 h and at low temperature the pellets of several batches were combined for one set of analyses. Since slight changes in culture conditions result in significant changes in pigmentation of the cells the data gained from one fragmentation can account for the average of the results of a series of different batches. From a number (3 to 4) of fragmentations of cells grown under the different conditions the data of those fractions were selected which appeared to be the most purified ones.

The isolated and purified cell fractions first were examined for their morphology by electron microscopy as negatively stained preparations and the characteristic absorption spectra of the fractions were recorded (Figs 1 – 4). As can easily be recognized from the micrographs (Fig. 1 – 3) the fractions corresponding to chlorosomes exhibited significant differences in their morphology when isolated from cells grown under the

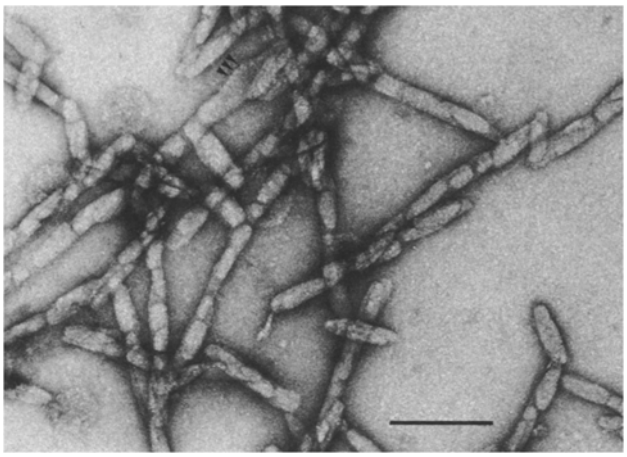

Fig. 1. Isolated chlorosomes from *Chloroflexus aurantiacus* Ok-7o-fl grown at 50° C and 400 lux (low light-cells). Arrows mark an area of periodic fine structure on a chlorosome that was flattened during the preparation. The pattern equals that of the "baseplates" which can be recognized in "chlorosome bags" of BChl *c*-depleted cells (Figs. 2B and 3C). Bar indicates 200 nm

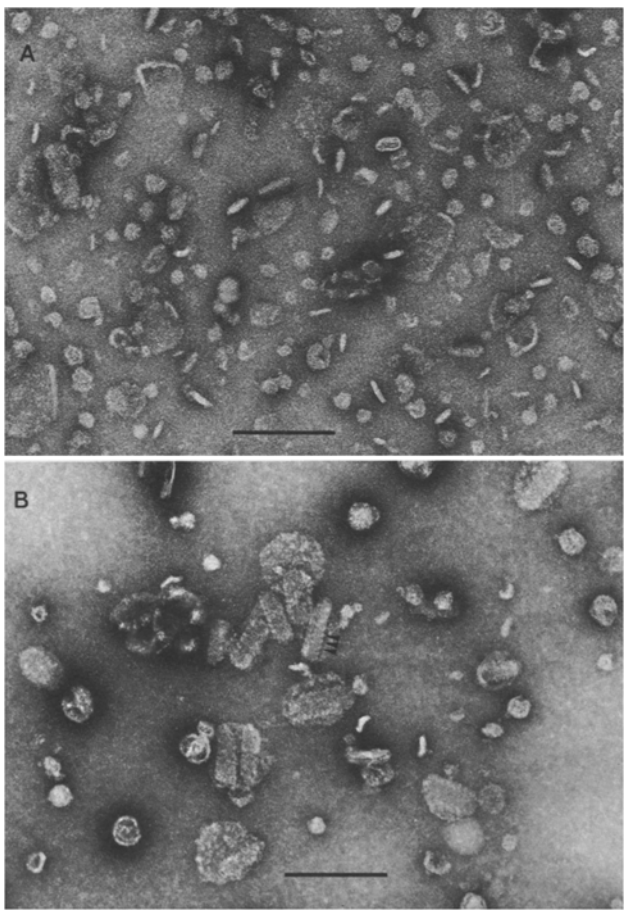

Fig. 2A and B. Isolated cell fragments from *Chloroflexus aurantiacus* Ok-7o-fl grown at 50° C and 2,000 lux (high light-cells). (**A**) Band a of the sucrose gradient (see Fig. 4). (**B**) Band c. Arrows mark the periodic substructure of "chlorosome bags". Bars indicate 200 nm

various growth conditions. As was demonstrated before (Schmidt, 1980) isolated chlorosomes from low light-cells were long cigarre like bodies of about 35 × 150 nm in size. When undamaged they did not show any visible fine structure on the surface (Fig. 1). The cytoplasmic membrane fraction of these cells (not shown here) consisted of flat pieces of membrane fragments, mostly covered with regularly formed arrays of surface structures, perhaps enzyme structures.

The fractions showed characteristic absorption spectra in the BChl-region (Fig. 4A, gradient bands a and c, Table 1). The chlorosome fraction (band a) was characterized by a very intense peak at 740 nm which almost covered the chlorosome specific BChl *a*-peak at 790 nm. The cytoplasmic membranes (band c), in contrast, contained all of the BChl *a*-complexes with absorption maxima at 808 and 866 nm. But still a considerable amount of BChl *c* (740 nm) was present, probably caused by impurities with a few remaining chlorosomes. As the ratio of chlorosomes : cytoplasmic membranes in the mixed fractions b_1 and b_2 decreased peak II (790 – 808 nm) increased in magnitude and shifted towards 804 nm. Peak I (866 nm) at the same time became more prominent and peak III (740 nm) decreased as can be seen from the peak ratios in Table 1. Corresponding fractions isolated from cells with low BChl-content (high light-cells and low temperature-cells) differed significantly from those described above. In the 25/30 % layer of the sucrose gradients (band a) mainly small particles were accumulated accompanied only by a very few chlorosome-like bodies and some fragments of cytoplasmic membranes (Fig. 1A). On micrographs (Fig. 3A and B) the particles looked like irregular aggregates of protein structures rather than vesicles formed by membrane fragments. Many of these particles were of about the same diameter as chlorosomes. Although hardly any "intact" chlorosome could be identified in these bands these fractions exhibited absorption spectra characteristic of fully developed purified chlorosomes from low light-cells (Fig. 4B, bands a and b, Fig. 4A, band a). It therefore was assumed that the accumulated particles carry the bulk of the BChl *c* and the 790 nm-BChl *a*-component.

The fraction of cytoplasmic membranes (40 – 45 % sucrose) of the gradient (Fig. 4B, band c) was composed of flat round pieces of membrane fragments and a number of particles similar to those found in the upper layers (Fig. 2B). Most of the membrane fragments carried one or two chlorosome-like structures characterized by a series of periodically arranged substructures (Fig. 2B, black arrows). These bodies in general had the same width as had the isolated chlorosomes from low light-cells. They mostly were much shorter and not ellipsoid at the ends, appearing as if the tips were broken off the bodies.

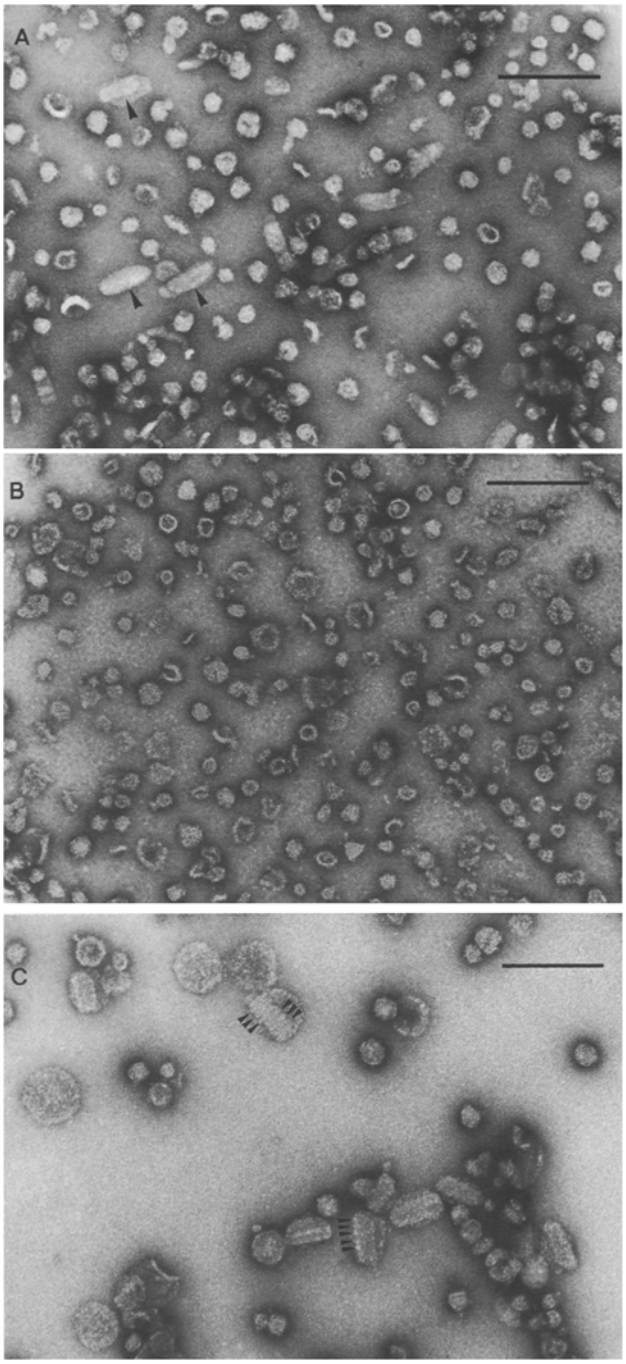

Fig. 3A—C. Isolated cell fragments from *Chloroflexus aurantiacus* Ok-7o-fl grown at 38—39° C and 1,500 lux (low temperature-cells). **(A)** Band a of the sucrose gradient (see Fig. 4). **(B)** Band b. **(C)** Band c. Arrows point to chlorosome structures **(A)** and periodic substructures of "chlorosome bags" **(C)**. Bars indicate 200 nm

The prominent peak in the absorption spectra of this band was at 866 nm (peak I, Fig. 4B). Although there was a considerable number of chlorosome-like structures associated with the isolated membrane fragments the BChl *c*-peak at 740 nm was very low as compared to the spectra of band c from low light-cells. This may indicate that most of the BChl *c* was accumulated with the fragments in the upper layer of the gradient and that the bodies on the cytoplasmic membranes can be considered as to be BChl *c*-depleted "chlorosome bags".

The fragments isolated from low temperature-cells revealed about the same morphological structures and absorption spectra as the corresponding fractions obtained from high light-cells. It seemed, however, that the membranes of these cells were more fragile. It thus was impossible to get the same degree of purification of fragments. The spectra of band a (Fig. 4C) always had a small but detectable elevation around 860 nm. This fraction consisted mainly of the particles described for high light-cells, mixed with a few chlorosome structures mostly attached to small fragments of cytoplasmic membranes (Fig. 3A, arrows). The isolated band b (Fig. 3B) in its major part was made up by the particles of different sizes, almost free of chlorosome structures but mixed with a few more membrane fragments than band a. The fraction of cytoplasmic membranes from low temperature-cells was almost indistinguishable from that isolated from high light-cells. But it seemed that the length of most of the "chlorosome bags" was shorter (Fig. 3C). In contrast to the absorption spectra of band c from 2,000 lux-cells in the spectra of low temperature-membranes (band c, Fig. 4C) the 740 nm peak was more reduced (see also peak ratios in Table 1). These differences in the spectra of the cytoplasmic membrane fractions of the two preparations were in good agreement with the different length of "chlorosome bags" if it is assumed that BChl *c*-occurrence is strictly bound to the presence of chlorosomes.

After 24 h of reactivation of BChl *c*-synthesis "complete" chlorosomes appeared in the 25/30 % layer of the sucrose gradients again. But many of the membrane fragments in band c carried the same "chlorosome bags" as were found in the initial cells. The spectra of this fraction, however, showed an increased BChl *c*-peak at 740 nm (Fig. 4D, Table 1), indicating that BChl *c*-synthesis in the bags takes place probably directly in connection with the cytoplasmic membrane.

Pigmentation

All membrane fractions gained from cells grown under the different growth conditions mentioned above were analysed for their specific pigment content and composition. The data of these analyses confirmed the results obtained from electron microscopic control and absorption spectra described above. Table 2 summarizes the carotenoid, BChl *c*, and BChl *a* contents and their molar ratios. It was found that in high light-cells mainly the BChl *c*-content is reduced, whereas the

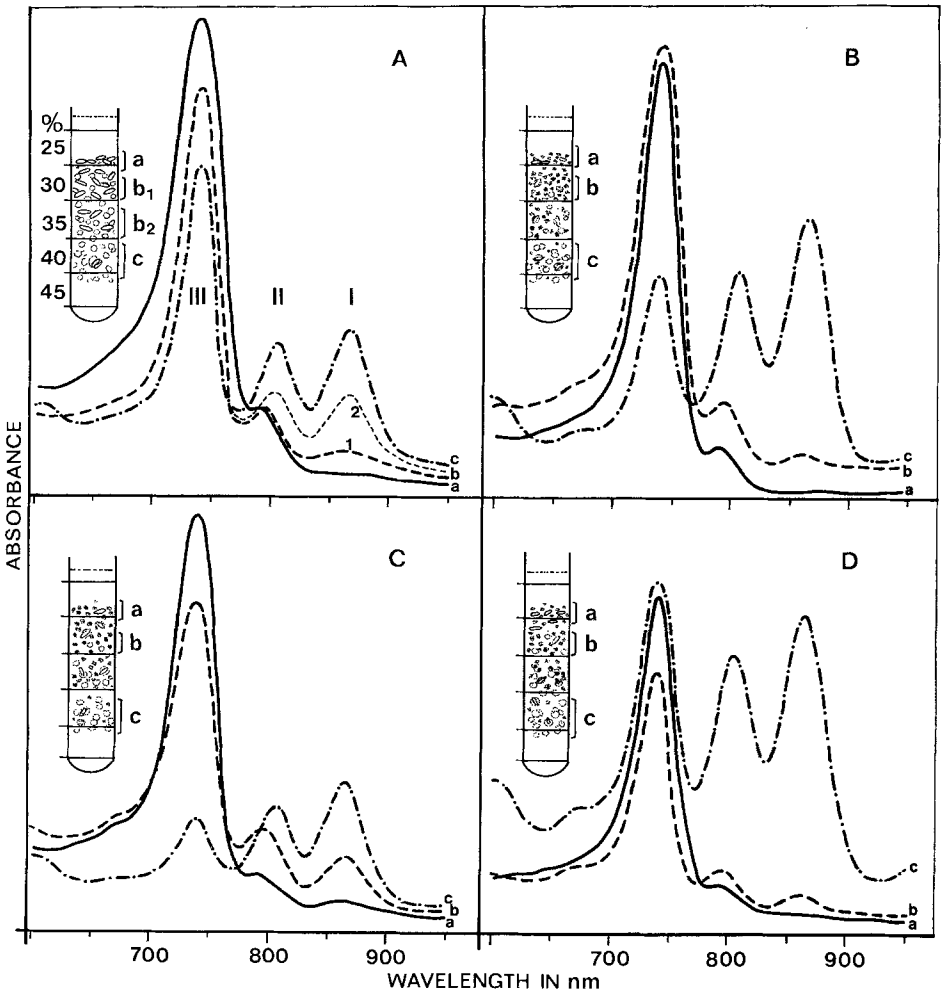

Fig. 4. Absorption spectra of the chlorophyll components of isolated cell fragments from *Chloroflexus aurantiacus* Ok-7o-fl

amount of BChl *a* was only slightly changed in crude extracts as compared to the pigment content in low light-cells. Also the carotenoid content remained almost unchanged. But as in low light-cells the bulk of BChl *c* was accumulated in band a (about 93 % of total BChl) although this fraction consisted almost entirely of particles (protein aggregates?). This fraction also was enriched in carotenoids. In contrast, the membrane fragments in band c (including "chlorosome bags") were characterized by a very low BChl *c* and carotenoid content, but the amount of BChl *a* was relatively high (56 % of total BChl).

In comparing the data of pigmentation of low temperature-cells with those from high light- and low light-cells it became obvious that low temperature did not only inhibit growth but also the synthesis of all types of pigments (carotenoids, BChl *c* and BChl *a*). The specific pigment content of the crude extract was extremely low. But again, the bulk of BChl *c* and the highest carotenoid concentration were found in band a (95 % of total BChl). The cytoplasmic membranes (band c) of these cells contained only half of the BChl (*a* and *c*) as was found in the corresponding fraction of high light-cells, the proportion of both components, however, was the same as in the cytoplasmic membrane of 2,000 lux-cells. The carotenoid content in cytoplasmic membranes was about the same in both types of cells. There was, however, considerably less carotenoid in the fraction of band a of 39° C-cells than in all other preparations. This may indicate that the formation of pigment complexes associated with carotenoid synthesis in the light-harvesting system was affected by growth at low temperatures.

In cells which were first grown at 39° C and then incubated for 24 h at 50° C and 400 lux there was an increase in the specific content of all three components of photopigments (carotenoids, BChl *c*, and BChl *a*). The analytical data of the isolated fragments showed that the carotenoid and BChl-contents in band a was about twice that of the initial cells. As mentioned before, this fraction contained an increased number of chlorosomes. Thus the rise in pigmentation apparently

Table 1. Absorption maxima and peak ratios of chlorophyll components in isolated fractions from *Chloroflexus aurantiacus* Ok-7o-fl grown under different growth conditions

Growth condition	Fraction[a]	Absorption maxima (nm)			Peak ratios (%)		
		I	II	III	I/III	II/III	I/II
50°C, 400 lux, 96 h	band a	–	(792)	740	5	18	26
(low light-cells)	b_1	(860)	794	740	10	21	51
	b_2	866	802	740	30	30	98
	c	866	806	740	51	46	110
50°C, 2,000 lux, 48 h	band a	–	792	740	3	14	21
(high light-cells)	b	860	795	740	4	16	25
	c	866	807	740	102	128	125
38–39°C, 1,500 lux, 8 d	band a	(860)	790	740	7	13	55
(low temperature-cells)	b	864	794	740	22	24	92
	c	866	806	740	139	116	121
38–39°C, 1,500 lux, 8 d	band a	–	790	740	7	14	46
→ 24 h, 50°C, 400 lux	b	864	794	740	13	21	62
	c	866	805	740	90	78	114
38–39°C, 1,500 lux, 8 d	band a	–	792	740	2	11	14
→ 48 h, 50°C, 400 lux	b	866	796	740	8	13	61
	c	866	806	740	78	65	120

[a] See bands in sucrose gradients in Fig. 4

Table 2. Pigment contents of isolated fractions from *Chloroflexus aurantiacus* Ok-7o-fl grown under different growth conditions

Growth condition	Fraction	Carot.	BChl *c*	BChl *a*	total BChl	BChl *c*	BChl *a*	BChl *c*: BChl	Carot: total BChl
		in μg/mg protein				% of total		molar ratio	
50°C, 400 lux, 96 h	crude extract	17	69	11	80	86	14	6.27	0.22
(low light-cells)	band a	33	348	18	366	95	5	19.33	0.09
	c	17	31	24	55	56	44	1.92	0.31
50°C, 2,000 lux, 48 h	crude extract	14	18	8	26	69	31	2.25	0.81
(high light-cells)	band a	30	93	7	100	93	7	13.29	0.45
	b	14	38	5	43	88	12	7.60	0.33
	c	9	8	10	18	44	56	0.80	0.78
38–39°C, 1,500 lux, 8 d	crude extract	5	2	1	3	67	33	2.00	2.50
(low temperature-cells)	band a	13	54	3	57	95	5	18.00	0.34
	b	9	18	3	21	86	14	6.00	0.64
	c	8	4	5	9	44	56	0.80	1.33
38–39°C, 1,500 lux, 8 d	crude extract	15	28	8	36	78	22	3.50	0.42
→ 24 h, 50°C, 400 lux	band a	25	106	7	113	94	6	15.14	0.22
	b	12	34	5	39	87	13	6.80	0.46
	c	13	12	6	18	67	33	2.00	1.08
38–39°C, 1,500 lux, 8 d	crude extract	18	36	10	46	78	22	3.60	0.78
→ 48 h, 50°C, 400 lux	band a	18	95	8	103	92	8	11.88	0.26
	b	18	76	10	86	88	12	7.60	0.31
	c	10	14	10	24	58	42	1.40	0.63

Table 3. Carotenoid composition of isolated fractions from *Chloroflexus aurantiacus* Ok-7o-fl grown under different growth conditions

Growth condition	Fraction	Carotenoid composition (% of total)									
		Precursors[a]	Lycopene	γ-Carotene	β-Carotene	Oxo-carotenoids[b]	Rhodopin	OH-γ-Carotene	total Glucosides	Rhodopin-glucoside	γ-Carotene-glucoside
50°C, 400 lux, 96 h	crude extract	–	9	39	24	4	–	5	20	–	20
(low light-cells)	band a	–	4	43	36	5	–	3	9	–	9
	c	–	3	34	12	9	–	6	36	–	36
50°C, 2,000 lux, 48 h	crude extract	–	2	30	26	4	–	5	33	–	33
(high light-cells)	band a	–	8	38	30	5	–	4	15	–	15
	b	–	10	25	24	7	–	5	29	–	29
	c	–	1	23	17	11	–	5	43	–	43
38–39°C, 1,500 lux, 8 d	crude extract	9	2	16	11	3	–	5	52	–	52
(low temperature-cells)	band a	12	9	16	16	9	–	5	33	–	33
	b	7	3	14	14	10	–	5	46	–	46
	c	8	–	11	11	8	–	5	56	–	56
38–39°C, 1,500 lux, 8 d	crude extract	4	6	35	13	2	–	5	35	–	35
→ 24 h, 50°C, 400 lux	band a	3	44	23	7	2	5	–	16	9	7
	b	6	10	32	12	4	–	5	31	–	31
	c	–	tr	27	23	8	–	5	40	–	40
38–39°C, 1,500 lux, 8 d	crude extract	–	4	34	25	3	–	4	30	–	30
→ 48 h, 50°C, 400 lux	band a	–	4	41	34	3	–	3	15	–	15
	b	–	2	37	29	4	–	4	26	–	26
	c	–	tr	30	18	5	–	4	43	–	43

[a] ζ-Carotene and neurosporene
[b] 4-Oxo-γ-carotene and 4-oxo-β-carotene (echinenone)
tr: Traces

is due to an increase in more completely developed chlorosomes. In the cytoplasmic membranes (band c) also an increase in BChl *c* and carotenoids was found, the BChl *a* content, however, was not significantly changed. These findings suggested that the incubation at low light and high temperature resulted in completing the light-harvesting system in the preformed sites, i.e. "chlorosome bags", at the cytoplasmic membranes, causing the occurrence of chlorosomes in the upper layers of the sucrose gradient.

The carotenoid composition of chlorosomes and cytoplasmic membranes of low light-cells is very specific (Schmidt, 1980). The main components of chlorosomes are γ- and β-carotene. The cytoplasmic membranes contain a high percentage of γ-carotene glucoside in addition to γ-carotene and some β-carotene (Table 3). The carotenoid analyses of fragments from high light- and low temperature-cells revealed that the carotenoid composition was strikingly changed. The content in the glucosidic component was increased, especially in low temperature-cells, apparently mainly due to the high concentration of this compound in the cytoplasmic membranes.

Although there was more glucosidic carotenoid present in high light-cells the proportion of the individual carotenoids resembled that of low light-cells in all fractions isolated. There was, however, a remarkable difference in carotenoid composition of low temperature-cells. Neurosporene and ζ-carotene, the more saturated precursors of lycopene, were accumulated. Very often lycopene, the aliphatic precursor of γ-carotene, and rhodopin were synthesized rather than γ- and β-carotene. This indicated that during growth at low temperature not only the synthesis of total carotenoids was reduced but also some steps of the biosynthetic pathway (inchain desaturation and cyclization) were specifically inhibited. After incubation of these cells at 50°C and 400 lux for 24 h the amount of glucosidic carotenoids and the accumulation of the early precursors was slightly reduced. The content in γ-

Table 4. Phospholipid, glycolipid, cytochrome *c* contents, and succinate dehydrogenase activity of isolated fractions from *Chloroflexus aurantiacus* Ok-7o-fl grown under different growth conditions

Growth condition	Fraction	Phospho-lipids	Glyco-lipids[a]	Cytochrome *c*	Succinate dehydrogenase
		µg/mg protein		µmol/g protein	nmol/mg protein × min
50°C, 400 lux, 96 h	crude extract	37.0	40.0	1.47	38.4
(low light-cells)	band a	62.5	106.0	0.10	–
	c	37.5	36.0	3.43	60.7
50°C, 2,000 lux, 48 h	crude extract	39.6	37.3	0.90	42.6
(high light-cells)	band a	135.6	68.9	0.16	–
	b	75.4	55.6	0.17	20.8
	c	44.1	26.9	1.08	72.0
38–39°C, 1,500 lux, 8 d	crude extract	16.3	15.8	0.11	25.6
(low temperature-cells)	band a	93.2	96.4	–	1.4
	b	48.8	38.6	0.59	80.8
	c	33.0	7.1[b]	0.94	91.2
38–39°C, 1,500 lux, 8 d	crude extract	37.0	25.5	1.12	19.8
→ 24 h, 50°C, 400 lux	band a	174.0	91.3	0.35	0.2
	b	105.8	43.6	0.58	24.5
	c	45.0	15.7	1.92	57.4
38–39°C, 1,500 lux, 8 d	crude extract	49.7	33.9	1.51	55.4
→48 h, 50°C, 400 lux	band a	193.8	57.0	0.16	n.d.
	b	119.4	65.2	0.88	30.1
	c	49.0	22.8	1.38	55.5

[a] Determined as hexose from lipid extracts
[b] In other preparations values were higher
n.d.: not determined

and β-carotene increased instead concomitantly with the increase of BChl *c*. After 48 h of incubation the same proportion as found in low light-cells was reached again, although the specific BChl *c*-content was considerably lower (Table 2).

From the distribution of the individual carotenoid components within the fractions and changes in composition in connection with BChl *c*-synthesis it is concluded that the major part of the γ- and β-carotene is a component of the light-harvesting system of the photosynthetic apparatus in *Chloroflexus*. Simultaneously with the filling of "chlorosome bags" with BChl *c*, as suggested before, the content of γ- and β-carotene in the cytoplasmic membrane was increased, causing an apparent decrease in the amount of glucosidic carotenoids. From these analyses no suggestions could be made which components of the carotenoids found in the cytoplasmic membranes function as members of the reaction centers. But from analyses of carotenoids of aerobically grown *Chloroflexus* cells (Schmidt, 1976) it seems that at least most of the glucosidic compounds are part of the membrane itself rather than of the photosynthetic apparatus located in it.

Other Components

For identifying the origin of the fragments obtained from cells grown under the different growth conditions, it was necessary to look for their content of phospholipids, glycolipids, cytochrome *c*, and activity of succinate dehydrogenase. These components are characteristically distributed in the isolated chlorosomes and cytoplasmic membranes (Schmidt, 1980). Table 4 summarizes the analytical data. It was found that bands *a* from high light- and low temperature-cells corresponding to purified chlorosomes from low light-cells accumulated the bulk of phospho- and glycolipids even though there were only very few chlorosome structures present. It also could be observed that the phospholipid content, based on mg of protein, in these fractions was much higher when compared with the data from purified chlorosomes of low light-cells. There was little, if any, cytochrome *c* and succinate dehydrogenase activity detected. Small amounts and low enzyme activities sometimes observed may originate from fragments of the cytoplasmic membranes still present. The analytical data (high BChl *c*, phospholipid, and glyco-

lipid contents, little or no cytochrome *c* and succinate dehydrogenase activity) suggested the band *a*-particle fractions consist of an accumulation of destructed "chlorosome bags" accompanied by the bulk of their light-harvesting pigment protein complexes. These fractions are also rich in glucosidic carotenoids, the characteristic carotenoid component of the cytoplasmic membrane. This fact indicates that there must be a close relationship between baseplates of chlorosomes, phospholipid layer and attachment site of the cytoplasmic membrane. This interpretation would be in good agreement with the chlorosome model recommended by Staehelin et al. (1978, 1980).

Electrophoresis

Preliminary SDS-electrophoresis revealed some typical components of isolated chlorosomes and cytoplasmic membranes. The approximate molecular weights of proteins from chlorosomes, 10,000 and 15,000, were identical with the characteristic proteins of light-harvesting systems (Drews, 1978; Thornber et al., 1978; Olson, 1978). In addition two more bands of 40,000 and 57,000 dalton were present. The 40 kdalton band probably resembles that of the BChl *a*-trimer-protein from *Chlorobium* (Olson et al., 1976a, b; Olson, 1978). But no conclusion at this time could be drawn with respect to the 57 kdalton protein.

The characteristic proteins obtained from cytoplasmic membranes from *Chloroflexus* exhibited molecular weights of about 24,000, 27,000, 29,000 ($\pm$ 2) and some bands with higher molecular weights, between 60 and 85 kdalton. Also the 12.5 and 15 kdalton and the 40 kdalton proteins were still present but in significantly reduced concentrations. The 24, 27 and 29 kdalton bands seemed to be identical with the reaction center proteins from phototrophic purple bacteria (Drews, 1978; Thornber et al., 1978). But more detailed analyses have to be made to confirm this suggestion.

When the corresponding fractions from high light- and low temperature-cells were analyzed for their protein composition it was found that they did not differ from purified chlorosomes and cytoplasmic membranes from low light-cells. Occasionally, some weak bands typical of cytoplasmic membrane proteins were observed in gels of the chlorosome corresponding fractions, as well indicating that the separation was not always perfect. With increasing amounts of fragments of cytoplasmic membranes (30 + 35% sucrose), however, the concentration of characteristic membrane proteins increased. This is in good agreement with the analytical data (Table 2 – 4) of these fractions.

Discussion

On the basis of similarities of cytology and absorption spectra, i.e. the presence of chlorosomes and BChl *c* as the predominant chlorophyll-component, it was assumed that the photosynthetic apparatus of Chloroflexaceae and Chlorobiaceae were functioning in about the same way. The description of isolated reaction center components from *Chlorobium limicola* K2 (Sybesma and Olson, 1963; Olson et al., 1976a; Whitten et al., 1979) and the studies on pigmentation of *Chloroflexus* in relation to growth (Pierson and Castenholz, 1974) clearly demonstrated that the organization of the photosynthetic apparatus of both types of organisms differs profoundly. This was also confirmed by a comparative study on the chemical composition of isolated membrane fractions from *Chloroflexus* Ok-70-fl and *Chlorobium limicola* 6230 (Schmidt, 1980).

As in Chlorobiaceae the components of the photosynthetic apparatus in *Chloroflexus* are separately localized on two cytological different parts of the cell: the light-harvesting pigment system, consisting of BChl *c*, is found in the chlorosomes. The reaction center (RC) and light-harvesting (LH) BChl *a*-complexes are exclusively situated in the cytoplasmic membrane. In contrast to the findings in *Chlorobium*-membranes the BChl *a*-components in *Chloroflexus*-membranes exhibit pronounced peaks at 808 and 866 nm, the peak ratio of which is almost constant in absorption spectra of cytoplasmic membranes from cells of all growth conditions (Table 1, Fig. 4). This fact suggests that the synthesis of these two complexes (LH-BChl *a* and RC-BChl *a*) in the cytoplasmic membrane is regulated in concert. Thus, this part of the photosynthetic unit probably is of the same type as the RC/B875-subunit from chromatophores of *R. rubrum* and *R. sphaeroides* (Aargard and Sistrom, 1972; Drews, 1978; Sistrom, 1978; Thornber et al., 1978). The BChl *c*-component then could be considered as to be an analogue to the B850/800-complex of *R. sphaeroides* which varies the size of the photosynthetic unit. From this point of view it can be stated that the photosynthetic unit of *Chloroflexus* resembles that of purple bacteria rather than that of the Chlorobiaceae.

The regulation of synthesis of the light-harvesting pigment system localized in the chlorosomes is much more sensitive to alterations of growth conditions than that of the pigment system of the cytoplasmic membranes. The extent of variation in BChl *c*-content is incomparably larger in cells of *Chloroflexus* than in *Chlorobium* species (Pierson and Castenholz, 1974; Broch-Due et al., 1978). When light intensities were increased and/or temperature decreased the cells responded with a remarkable decrease in BChl *c*-content. The lowest amount measured was 0.63 µg BChl *c* per mg of dry weight (ca. 1.2 µg/mg protein) at 54,000 lux (Pierson and Castenholz, 1974). In their paper the authors suggested that the BChl *c*-content is regulated

by the number of chlorosomes present in the cells. This would presume that existing chlorosomes contain the same amount of BChl *c* under all growth conditions.

On electron micrographs (not shown in this paper) of ultrathin sections of *Chloroflexus* cells grown at 50° C and 400 lux (low light-cells) chains of chlorosomes of different length could be recognized. The site of attachment at the cytoplasmic membrane was clearly marked by an electron dense layer. In sections of cells grown at high light or low temperature (both types low in BChl *c*-content) quite a large number of these electron dense layers was present. But they appeared to be considerably shorter. Chlorosome structures could never be visualized in those thin sections, though. On isolated cytoplasmic membranes, however, numerous shortened chlorosome-like structures could be identified attached to small membrane fragments.

From these observations and the analytical data on the chemical and pigment composition it was concluded that the structures found in connection with the cytoplasmic membranes in low temperature- and high light-cells were preformed "chlorosome bags" the basic part of which could be seen as a periodic fine structure (Figs. 2B, 3B). It seems to be identical with the baseplate of *Chlorobium* chlorosomes as was recently proposed by Staehelin et al. (1980). This part of the "chlorosome bags" must be in close connection with the cytoplasmic membrane, or even part of it. Apparently, it is identical with the electron dense layer seen in thin sections. This part of the membrane is apparently very fragile and sensitive to the methods of breakage, as were the "chlorosome bags". Together with fragments of "chlorosome bags" this lipid-layer accumulated as particles in the upper layers of the sucrose gradients (25 – 30 %) causing a pronounced peak at 790 – 792 nm. This fact suggested that the phospholipid-rich area of the attachment site contains the 790 nm-BChl *a*-complex. In these 25 – 30 %-layers of the gradients the specific content of BChl was reduced but the proportion of both, BChl *c* and BChl *a*, was the same as in the corresponding fraction with intact chlorosomes of low light-cells (Table 2), indicating that the fragments were derived from destructed chlorosome structures. Since the specific BChl *c*-content was highly enriched with these fragments it can be concluded that the "chlorosome bags" in BChl *c*-depleted cells still contain a certain amount of this pigment. This can be stated also from the results of Pierson and Castenholz (1974) and N. B. Pellerin (1979, unpublished). Most of the BChl *c* obviously is released with the fragments during the breakage of cells and the sonification of cell fragments prior to the gradient centrifugation.

When BChl *c*-synthesis was reactivated by incubating low temperature-cells at 50° C and 400 lux for 24 h "complete" chlorosomes again accumulated with the 25/30 % sucrose-band. Simultaneously the BChl *c*-content in cells and isolated fractions increased considerably (two- or threefold in cells, twofold in band a, and sixfold in cytoplasmic membranes) although there was no or neglegible growth as measured by protein determination. The BChl *a*-content of cells was also increased. But this was mainly due to an increase of 790 nm-BChl *a* in band a. The BChl *a*-content of cytoplasmic membranes remained almost constant. These data led us to conclude that under conditions of impaired synthesis of light-harvesting pigments preformed "chlorosome bags" were present on their specific sites on the membranes. When the synthesis of these pigments was induced, first the "bags" were filled with the necessary light-harvesting pigment systems within the first 24 h before growth was continued. After 48 h of incubation, allowing a twofold increase of protein, there was a further increase in pigmentation and accumulation of "complete" chlorosomes.

We have not been able so far to achieve BChl-synthesis after growth of *Chloroflexus* cells in high oxygen tension. Structures attached to isolated cytoplasmic membrane fragments of aerobic cells looked completely different from those found in low temperature-cells. But areas with the same periodic fine structure as found in "chlorosome bags" were detected (unpublished observations). These are probably identical with the baseplates of chlorosomes. Further work remains to be done on these structures in order to find out whether they represent lipid areas without "bags" on them so that they can not be refilled with light-harvesting pigment systems under the appropriate conditions.

The data obtained in this study do not allow conclusions with respect to the definite fine structure of the chlorosomes and their attachment site on the cytoplasmic membrane. But in comparing the results given in this paper with the models of the chlorosome obtained from freeze fraction preparations by Staehelin et al. (1978, 1980) and Sprague et al. (1979) it can be suggested that a) chlorosomes of *Chloroflexus* strains are attached to the cytoplasmic membrane closely by a layer of pronounced periodic fine structure, which may as well be part of the membrane. b) This layer is rich in phospholipid and apparently contains the 790 nm-BChl *a*-complex. c) Chlorosomes, completely filled with light-harvesting pigments, are easily released from their site on the cytoplasmic membrane together with the phospholipid rich layer and the 790 nm BChl *a*. d) When *Chloroflexus* cells are depleted of the light-harvesting pigments, "chlorosome bags" are still attached to the surface of cytoplasmic membranes. These bags probably contain a small amount of BChl *c*. During the fragmentation and isolation procedures

these structures easily fall into small pieces which accumulate in the upper layer of sucrose gradients. The absorption spectra and the chemical composition of these fractions are almost identical with those of complete chlorosomes.

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