Arch. Microbiol. 124, 21 – 31 (1980)

Archives of
Microbiology

A Comparative Study on the Composition of Chlorosomes (Chlorobium Vesicles) and Cytoplasmic Membranes from *Chloroflexus aurantiacus* Strain Ok-70-fl and *Chlorobium limicola* f. *thiosulfatophilum* Strain 6230

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Abstract. Highly purified fractions of chlorosomes and cytoplasmic membranes were isolated from *Chloroflexus aurantiacus* Ok-70-fl and *Chlorobium limicola* 6230. These fractions were comparatively analyzed for their pigmentation, phospholipid, glycolipid, and cytochrome *c* content as well as for their specific activities of succinate dehydrogenase and NADH-oxidase. The data showed that there are some differences in pigmentation and phospholipid content between the isolated fractions of *Chloroflexus* and *Chlorobium*. Chlorosomes of *Chloroflexus* contained a specific BChl a-complex with a characteristic absorption maximum at about 790 nm. This BChl a-complex could not be detected in spectra of chlorosomes from *Chlorobium*. The near infrared region of the spectra of the isolated cytoplasmic membranes of both organisms revealed considerable differences: The BChl a-complexes of *Chloroflexus* membranes exhibited peaks at 806 and 868 nm whereas the membranes of *Chlorobium* had a single BChl a-peak at 710 nm. In contrast to the findings with *Chlorobium* the chlorosomes of *Chloroflexus* contained at least twice as much phospholipids as did the cytoplasmic membranes. In *Chlorobium* the phospholipid content of cytoplasmic membranes is three times that of their chlorosomes. The distribution of all other components (carotenoid composition, enzyme activities, cytochrome *c* content, and glycolipids) was about the same in both strains. From the data it was concluded that differences in the organization of the photosynthetic apparatus are mainly based on differences of the organization of the photosynthetic units in the cytoplasmic membrane and probably the kind of linkage of the light harvesting system in the chlorosomes with the reaction center in the cytoplasmic membranes.

Key words: *Chloroflexus aurantiacus* – *Chlorobium limicola* – Chlorosomes – Cytoplasmic membranes.

Abbreviations: BChl c = bacteriochlorophyll c; BChl a = bacteriochlorophyll a; DSM = Deutsche Sammlung von Mikrorganismen

The gliding, filamentous, photosynthetic bacterium *Chloroflexus aurantiacus* resembles the Chlorobiaceae with respect to cytological organization. Both types of organisms are characterized by their chlorosomes („Chlorobium vesicles"), small bodies, closely attached to the cytoplasmic membrane (Cohen-Bazire et al., 1964; Pierson and Castenholz, 1974a; Madigan and Brock, 1977; Staehelin et al., 1978). it seems to be confirmed now that in the Chlorobiaceae these vesicles carry the bacteriochlorophyll c (BChl c), the light harvesting system of the photosynthetic apparatus. Analyses of isolated reaction center complexes suggested that the reaction center and the components of the photoreactions are arranged in the cytoplasmic membrane (Olson et al., 1976a). A model of the construction and probable functions of the whole photosynthetic apparatus in *Chlorobium* cells was presented by Olson et al. (1976b). But there has been little information about the detailed characterization of the structure of isolated membrane types. A few analyses on the chemical composition of isolated chlorosomes of some *Chlorobium* strains have been published (Sykes et al., 1965; Schmitz, 1967; Cruden and Stanier, 1970). But only Cruden and Stanier looked for the composition of cytoplasmic membranes as well.

The claim that the photosynthetic apparatus of *Chloroflexus* strains is very likely the same as that of the Chlorobiaceae is mainly based on electron microscopic work and BChl determinations in whole cells (Pierson and Castenholz, 1974b; Gorlenko et al., 1975; Madigan and Brock, 1977). It therefore seemed to be important to isolate chlorosomes and cytoplasmic membranes from *Chloroflexus* cells for chemical

0302-8933/80/0124/0021/$02.20

analysis and for comparison with data gained from corresponding cell fractions from *Chlorobium limicola* f. *thiosulfatophilum*.

Materials and Methods

Organisms and Growth Conditions. *Chloroflexus aurantiacus* strain Ok-70-fl (DSM 636) was isolated by Pierson and Castenholz (1974a). Cells were grown in flat 250 ml screw-capped flasks at 50–52° C at 400 lux in a modified medium of Pierson and Castenholz, containing per 1000 ml of H_2O: 0.1 g Na_2HPO_4, 0.1 g $MgSO_4 \cdot 7\ H_2O$, 0.008 g NaCl, 0.1 g KNO_3, 0.7 g $NaNO_3$, 0.05 g $CaCl_2 \cdot 2\ H_2O$, 2 g Difco-yeast extract, 1.0 g glycyl-glycine (Fluka), 10 ml of a trace element solution (Pfennig and Lippert, 1966, without EDTA and iron citrate), 5 ml of an iron citrate solution, containing 100 mg Fe_3-citrate per 100 ml H_2O. pH was adjusted to 8.2–8.5 before sterilization. 250 ml were inoculated with 2.5 ml of a freshly grown culture and incubated for 5–6 days.

Chlorobium limicola f. *thiosulfatophilum* 6230 (DSM 249) was obtained from Dr. N. Pfennig's collection (Göttingen). Cells were grown in the medium of Biebl and Pfennig (1978), supplemented with 0.4% (w/v) Mg-acetate, 0.4% (w/v) Na-acetate, and 0.1% (w/v) $Na_2S_2O_3$ in a 10 l carboy with slow stirring at 28° C and 3000–8000 lux, depending on the density of the growing suspension. 10 l of medium were inoculated with 1.5 l of a freshly grown suspension of *Chlorobium*. Growth time was about 3 days.

Isolation of Chlorosomes and Cytoplasmic Membranes. Cells were harvested by centrifugation, washed and resuspended in 50 mM tris-HCl-buffer, pH 8.0, to a concentration of about 10–13 mg protein per ml. The cells were broken by passing the suspension two times through a precooled French pressure cell at 784,532N in the presence of DNase. Lysozyme and EDTA (50 µg/ml and 10^{-3} M, respectively) was added and the mixture incubated at 37° C for 30–45 min. Whole cells and large particles were sedimented by centrifugation at 12,000 × *g* for 15 min. The chlorosomes and membrane fragments were spun down at 14,000 × *g* for 2 h. The pellet was resuspended in the same buffer to a concentration of about 15–20 mg protein per ml. This suspension was treated with a weak ultrasonification for about 1 min directly prior to sucrose gradient centrifugation. Sonified suspensions were carefully mounted on discontinuous sucrose gradients (25–55% (w/w), Fig. 1). These were then centrifuged to equilibrium either in a SW 27 or SW 40 rotor at 24,000 rpm or 27,000 rpm in a Beckman Spinco L2 65B or Christ-Vacufuge, respectively. Bands were taken of the gradients with a Pasteur pipette. Corresponding fractions were pooled, freed from sugar by centrifugation after dilution with the buffer at 140,000 × *g* for 2 h and again purified by repeated centrifugation on sucrose gradients of the same concentrations.

Pigment Extraction and Determination. For estimations of total pigment content (BChl c, BChl a, carotenoids) 0.25 or 0.50 ml samples were extracted with 4.75 or 4.50 ml of methanol:acetone (1:1, v/v) for at least 60 min at room temperature under nitrogen in the dark. After a short centrifugation absorption spectra of the supernatant were run between 350 and 800 nm. Chlorophylls were estimated by using extinction coefficients given by Clayton (1963) for BChl a and Pierson and Castenholz (1974b) for BChl c. The amount of total carotenoids present was calculated by extrapolation from the absorption spectra at 460 nm using an average extinction coefficient $E^{1\%}_{1cm}$ of 3000. The individual carotenoids of the extracts were isolated and quantitatively determined after saponifying the extracts in the usual manner (Liaaen-Jensen, 1962, 1978). The unsaponified matter was separated on thin layer plates (Kieselgel G, Typ 60, Merck, Darmstadt) with 10%, 5%, and 2% (v/v) acetone in petroleum ether. Extinction coefficients employed for the calculation were adapted from Liaaen-Jensen et al. (1964), Davies (1965), and Halfen et al. (1972).

Cytochrome c. The cytochrome *c* content of the fractions was determined as alkaline pyridine hemochrome after extraction of most of the photopigments with methanol:acetone (2:7, v/v) according to Bartsch (1971, 1978) and Kakuno et al. (1971).

Phospholipids. Phospholipids were extracted with methanol:chloroform (2:1, v/v), as described by Bligh and Dyer (1959). The inorganic phosphorus was determined according to Boehringer Informations after ashing the dryed extract in 70% perchloric acid and a few drops of 30% H_2O_2 for 15 min at 180–200° C, using ammonium vanadate and ammonium molybdate as reagents. One mg phosphorus equals 25 mg of phospholipid (Miura and Mizushimo, 1968; Ketchum and Holt, 1970).

Glycolipids. The total content of glycolipids was determined as hexose with the anthrone method, using glucose as a standard (Hassid and Abraham, 1957). An aliquot of the lipid extract (see phospholipids) was taken to dryness under a stream of nitrogen and used for hexose estimation.

Protein. Protein was estimated by the modified Folin-Lowry method for whole cells (Lowry et al., 1951; Herbert et al., 1971).

Enzyme Activities. NADH-oxidase activity was measured by the method of Throm et al. (1970). Succinate dehydrogenase activity was assayed by the procedure of King (1963).

Absorption Spectra. Absorption spectra of whole cells and cell fractions were recorded in a Zeiss DMR 21 spectrophotometer, those of pigment extracts in a Unicam Sp 1800.

Results

Chloroflexus cells are very sensitive to any change of culture conditions. They respond to slight differences in light intensity with different growth rates and pigmentation (Pierson and Castenholz, 1974b, 1978). Variations in temperature also had striking effects on growth rate and pigmentation, including carotenoid biosynthesis and composition (Castenholz, personal communication; Schmidt, unpublished). Because of the quick and sensitive response of these organisms to small changes in growth conditions it was almost impossible to grow cells of the same composition and quality. The success of membrane and chlorosome isolation and purification, however, was highly dependent on the conditions during growth and these were difficult to control. The data in this study, therefore, are based on a series of analyses, the most representative of which are summarized in Tables 1–4 and Figs. 1–6.

Isolation of Cell Fractions. In order to obtain fractions of highly purified chlorosomes and cytoplasmic membranes it was necessary to repeat gradient centrifugation of the isolated bands two or three times. The yield was increased by a short and weak ultrasonic treatment of the suspension to be separated or purified. As is depicted in Fig. 1 purified chlorosomes of both strains studied are concentrated in the 25% layer. But in the case of *Chlorobium limicola* 6230 this band was split into two zones, one accumulating in the upper region and a second one being concentrated at the border line between 25 and 30% of sucrose. From electron microscopic preparations it was obvious that a network of threads, probably originating from the capsule of the outer cell layers, „contaminated" the upper chlorosome fraction. The lower density band consisted of a highly purified chlorosome suspension (Fig. 2B) just as the corresponding band from *Chloroflexus* cell extracts (Fig. 2A). In the latter case occasionally small fragments of membranes were found, but always present as less than 5%.

The two types of cytoplasmic membranes were enriched in different sucrose concentrations (Fig. 1). Cytoplasmic membranes of *Chloroflexus* cells accumulated in 40% sucrose, the most purified fragments at the border of 40–45% sucrose or in the upper 45% layer (Fig. 3A), whereas a completely pure membrane fraction from *Chlorobium* cells was found in the 35% layer of the gradient (Figs. 1 and 3B). This may be due to the very high phospholipid content of cytoplasmic membranes of *Chlorobium* strains compared to that of *Chloroflexus* (Table 4). There was also a remarkable difference in the formation of membrane fragments in both organisms as can be recognized by comparing Fig. 3A and 3B: cytoplasmic membranes from *C. limicola* accumulated as almost regular vesicles rather than flat pieces. Cytoplasmic membranes from *Chloroflexus* mostly formed flat fragments of irregular size which tended to stick together in clumps. Electron micrographs of negatively stained preparations indicated that the cytoplasmic membranes of both species were covered with regular arrays of protein structures. However, the protein structures in *Chloroflexus* preparations were half the size of those found on membrane surfaces of *Chlorobium*. Some of these large surface structures on *Chlorobium* membranes could often be seen attached to the surface of the chlorosomes. This may have lead Cruden and Stanier (1970) to the conclusion that the "chlorobium vesicles" do show a pronounced fine structure.

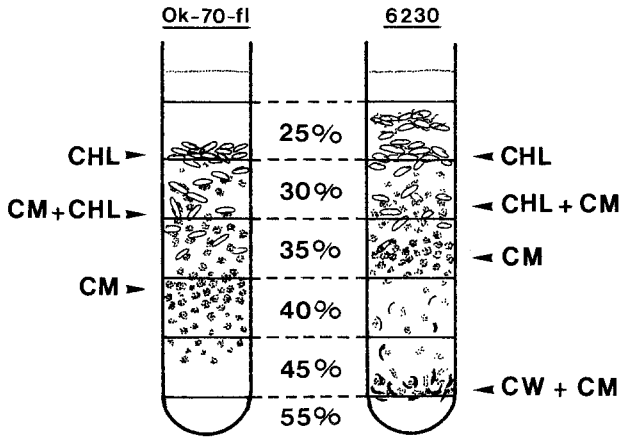

Fig. 1. Accumulation of cell fractions from *Chloroflexus aurantiacus* strain Ok-70-fl and *Chlorobium limicola* strain 6230 in a discontinuous sucrose gradient (25–55% sucrose in 0.05 M Tris-HCl-buffer, pH 8.0). *CHL* Chlorosomes, *CM* cytoplasmic membranes, *CW* cell walls

Absorption Spectra. The absorption spectra of whole cells of *Chloroflexus* Ok-70-fl and *Chlorobium* 6230 exhibited some differences especially in the BChl a-region (Fig. 4). *Chloroflexus* cells clearly showed peaks at 806 and 868 nm besides the BChl c-peak at 740 nm. In contrast a single BChl a-peak at about 810 nm in the spectrum of *Chlorobium* cells was reduced to a small inflection by the intense BChl c-peak at 754 m.

The differences between both organisms, however, became more pronounced in the spectra of the isolated chlorosomes and cytoplasmic membranes (Figs. 5 and 6). In chlorosome isolates from *Chloroflexus* Ok-70-fl no peaks at 806 or 868 nm could be detected but a shoulder at 790 nm in connection with the BChl c-peak. Since there was a small peak at 770 nm in methanol:acetone extracts of the chlorosomes, it was sup-

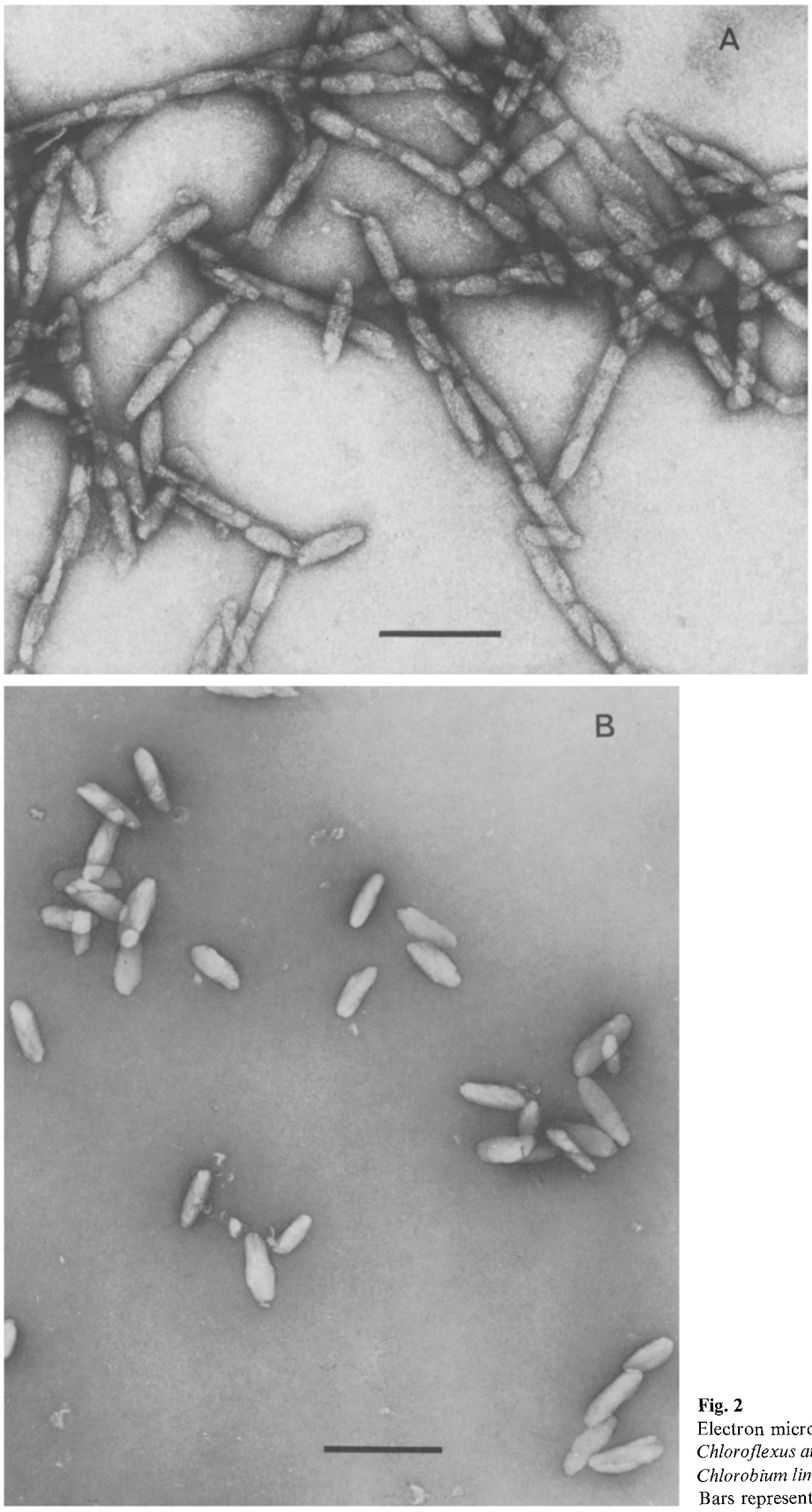

Fig. 2
Electron micrographs of chlorosomes isolated from *Chloroflexus aurantiacus* strain Ok-70-fl (**A**) and *Chlorobium limicola* strain 6230 (**B**)
Bars represent 200 nm (**A**) and 400 nm (**B**)

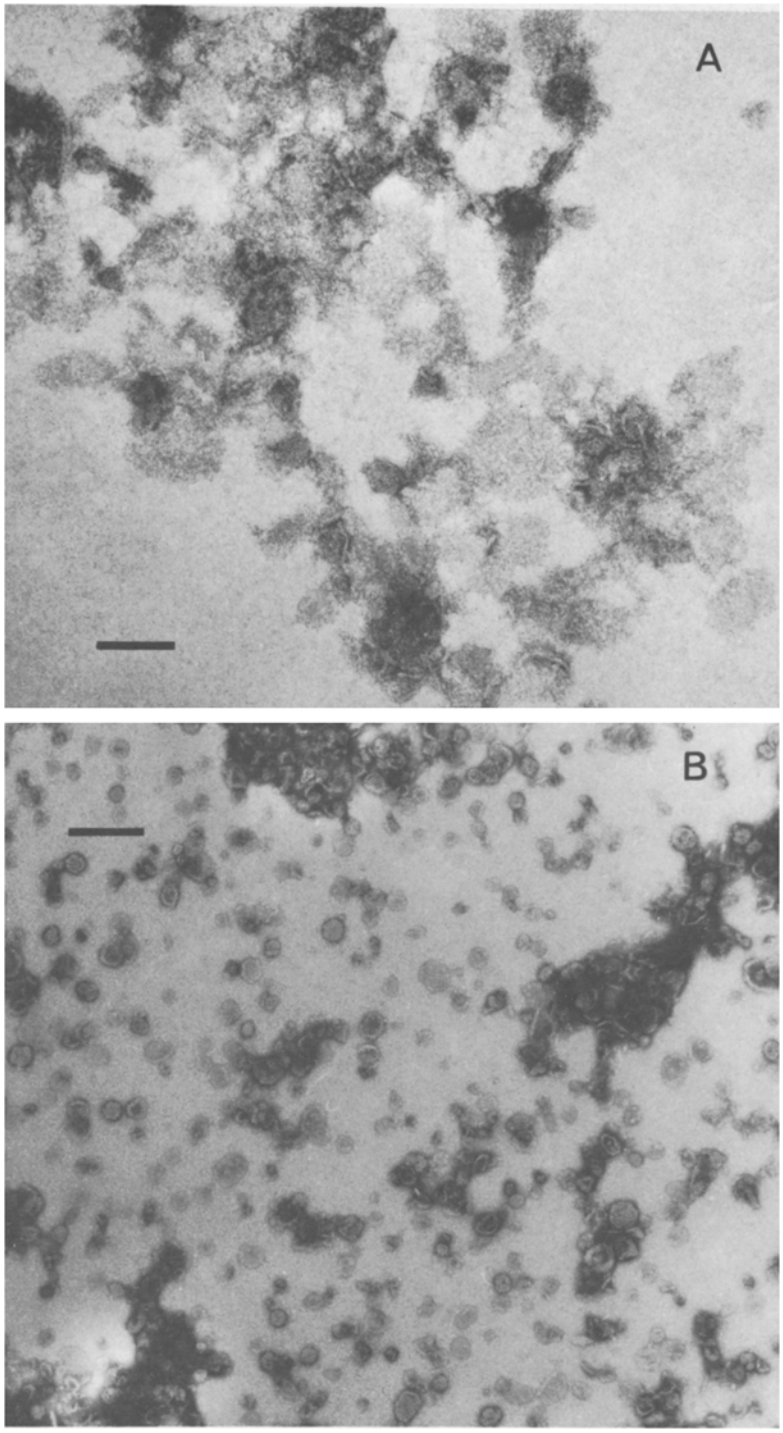

Fig. 3
Electron micrographs of cytoplasmic membranes isolated from *Chloroflexus aurantiacus* strain Ok-70-fl (**A**) and *Chlorobium limicola* strain 6230 (**B**). Bars represent 100 nm (**A**) and 200 nm (**B**)

posed that the 790 nm shoulder is due to a chlorosome specific BChl a-complex. A comparable inflection could not be identified in the spectra of chlorosomes from *Chlorobium* 6230, although there was a slight elevation at 770 nm in the spectra of extracts, indicating that there must be some BChl a present also.

In contrast, the BChl a-peaks in the spectra of cytoplasmic membranes of both organisms were very pronounced. In the case of *Chloroflexus* the 868 nm-complex (including the reaction center-BChl a, Pierson and Castenholz, 1974b) seems to be the main component. Never a sign of the 790 nm shoulder could be identified in these spectra, but always a remarkable amount of BChl c was still present. From the data obtained in this study it could not be concluded whether this BChl c is a component of the cytoplasmic membrane or whether, at least, part of it derived from a few chlorosomes still attached to the membranes.

From the data in Table 1 it became obvious that peak ratios of the three BChl-components are very characteristic of the isolated fractions from *Chloroflexus* and a good indication for the purification of the isolates. This was also expressed by the shift of peak II.

The absorption spectra of cytoplasmic membranes from *Chlorobium* cells differ considerably from those of *Chloroflexus* (Fig. 6). A fairly high BChl a-peak at 810 nm and a peak at 674 nm are typical of these spectra. No BChl c-peak at 754 nm could be detected in all preparations of cytoplasmic membranes of this strain. The absorption spectra of this fraction and its methanol:acetone extracts did not allow to draw any definite conclusion on the origine of the 674 nm-peak. But it is very likely that it derived from BChl c which was released from the chlorosomes during the isolation procedure and accumulated with the cytoplasmic membrane fraction in the sucrose gradient. A similar component was never seen in the corresponding fractions from *Chloroflexus* cells isolated under the same experimental conditions.

Pigment Composition. Table 2 summarizes the specific content of carotenoids and bacteriochlorophylls (BChl a and c) and their molar ratios in fractions from *Chloroflexus* Ok-70-fl and *Chlorobium* 6230. From the data it was obvious that the *Chlorobium* cells contained much more BChl c than *Chloroflexus*. The specific BChl a content, however, was about the same in both strains. Thus, the BChl c:BChl a ratio was much higher in all fractions from *Chlorobium* than in those from *Chloroflexus*. The same was true for the total BChl:carotenoid ratio because of the significantly high carotenoid content of *Chloroflexus* cells and fractions.

In both strains the chlorosomes contained a considerably higher amount of carotenoids than the cyto-

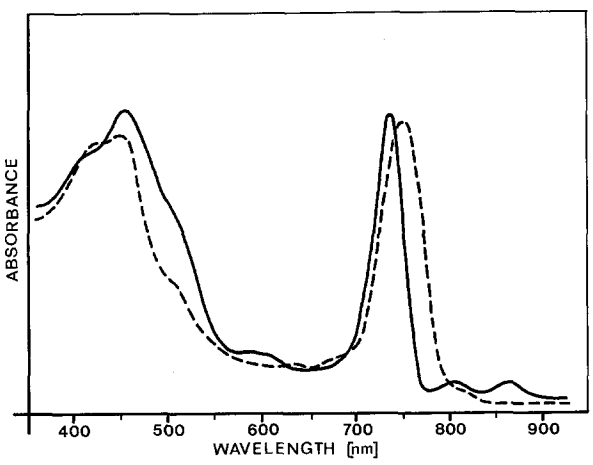

Fig. 4. Absorption spectra of whole cells of *Chloroflexus aurantiacus* strain Ok-70-fl, grown at 400 lux, 50° C (——) and *Chlorobium limicola* strain 6230 (– – –)

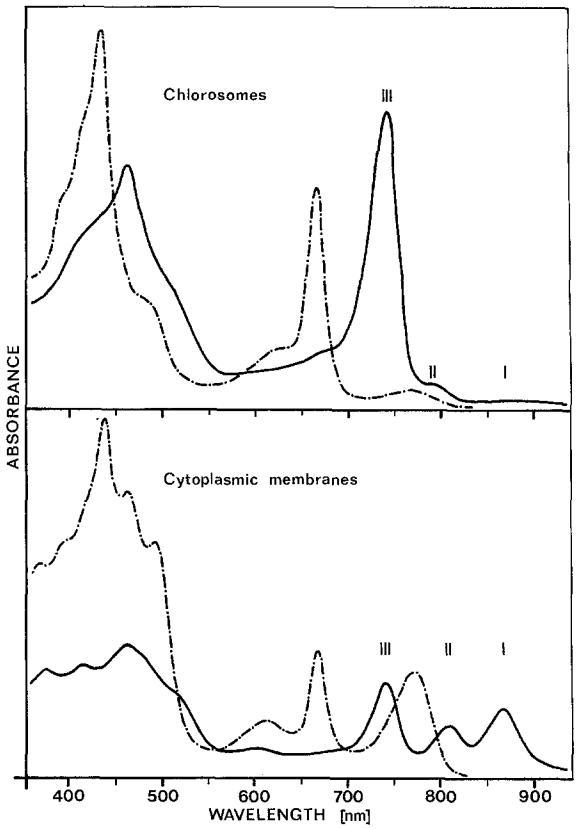

Fig. 5. Absorption spectra of isolated cell fractions from *Chloroflexus aurantiacus* strain Ok-70-fl. Suspension of fractions (——) and their methanol:acetone extracts (·—·), *I*, *II*, and *III* signing the three characteristic BChl-peaks

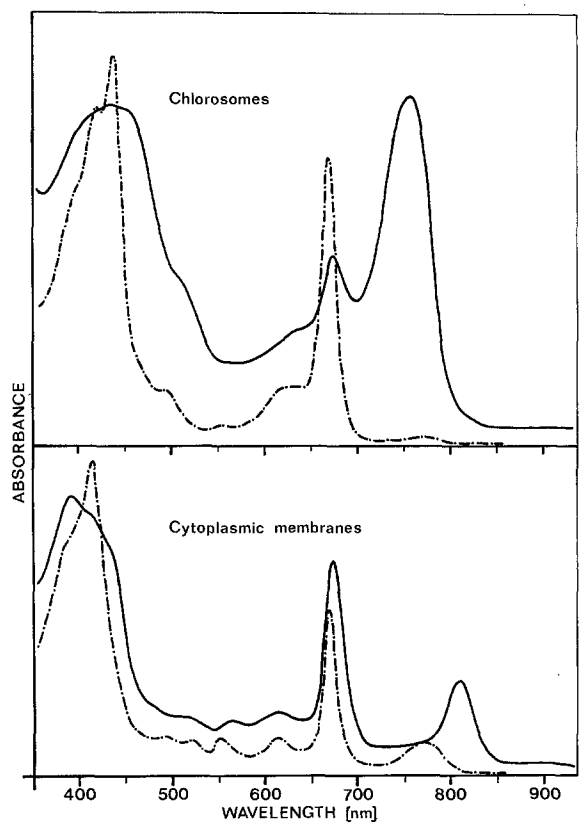

Fig. 6. Absorption spectra of isolated cell fractions from *Chlorobium limicola* strain 6230. Suspension of fractions (——) and their methanol:acetone extracts (·—·)

Table 1. Peak ratios of bacteriochlorophyll complexes in isolated chlorosomes and membranes from *Chloroflexus aurantiacus* Ok-70-fl

Fraction				Absorption maxima (nm)		
	% I/III	% II/III	% I/II	I[a]	II[a]	III[a]
Chlorosomes	3	8	31	–	790	740
Cytoplasmic membranes	72	56	128	868	808	740
Mixed fraction	27	27	98	868	802	740

[a] Symbols for the individual Bchl-peaks, see Fig. 5

Table 2. Pigment content of cell fractions from *Chloroflexus aurantiacus* Ok-70-fl and *Chlorobium limicola* f. *thiosulfatophilum* 6230

Fraction	in μg/mg protein				molar ratio	
	Carot.	Bchl c	Bchl a	total Bchl	Bchl c:Bchl a	Bchl:Carot.
Chloroflexus aurantiacus Ok-70-fl						
Broken cells	9	29	5	34	5.80:1	3.78:1
Crude extract	17	69	11	79	6.27:1	4.65:1
Chlorosomes	33	348	18	366	19.33:1	11.09:1
Cytoplasmic membranes	17	31	24	55	1.92:1	3.24:1
Chlorobium limicola f. *thiosulfatophilum* 6230						
Broken cells	7	251	22	273	11.41:1	26.00:1
Crude extract	7	310	13	323	23.85:1	30.76:1
Chlorosomes	19	583	18	601	32.39:1	21.09:1
Cytoplasmic membranes	2	78[a]	15	93	5.20:1	31.00:1

[a] This component probably is due to contaminations with Bchl c released from destroyed chlorosomes (Fig. 6)

Table 3. Carotenoid composition of the fractions from *Chloroflexus aurantiacus* Ok-70-fl and *Chlorobium limicola* f. *thiosulfatophilum* 6230

Carotenoid components	Fraction			
	Broken cells	Crude extracts	Chlorosomes	Cytoplasmic membranes
Chloroflexus aurantiacus Ok-70-fl				
Lycopene[b]	5[a]	9	4	3
γ-Carotene	37	39	43	34
β-Carotene	25	24	36	12
Oxo-components[c]	4	4	5	9
OH-γ-Carotene	4	5	3	6
OH-γ-Carotene-glucoside	25	20	9	36
Chlorobium limicola f. *thiosulfatophilum* (s. Table 4) 6230				
Neurosporene	17	17	21	5
Lycopene	4	4	8	trace
Chlorobactene	76	76	69	74
Rhodopin	<1	<1	trace	trace
OH-Chlorobactene	trace	<1	2	8
OH-Chlorobactene-glucoside[d]	trace	<1	<1	13

[a] Values are in % of total carotenoid content

[b] and [d] have not been described in these organisms before. The lycopene from *Chloroflexus* and Chlorobactene-glucoside were identified by chromatographic behaviour, acetylation, UV- and mass spectra (Schmidt, unpublished)

[c] 4-oxo-γ-carotene and 4-oxo-β-carotene (= Echinenone)

plasmic membranes. The carotenoid content of the membranes from *Chlorobium* seemed to be extremely low.

Not only did the quantity of carotenoids differ in both chlorosomes and cytoplasmic membranes, but also the proportion of the individual carotenoid com-

Table 4. Cytochrome c, glycolipid, phospholipid contents, and enzyme activities in fractions from *Chloroflexus aurantiacus* Ok-70-fl and *Chlorobium limicola* f. *thiosulfatophilum* 6230

Fraction	Cytochrome *c*	Hexose	P-lipid	NADH-Oxid	Succin.-DH[a]
	(μmol/g protein)	μg/mg Protein		nmol/mg protein × min.	
Chloroflexus aurantiacus Ok-70-fl					
Broken cells	0.69	22	18	1.1	20.5
Crude extracts	1.47	40	37	0.6	38.4
Chlorosomes	0.10	106	63	2.5	n.d.
Cytoplasmic membranes	3.43	36	37	1.5	60.7
Chlorobium limicola f. *thiosulfatophilum* 6230					
Broken cells	0.77	103	43	n.d.[b]	n.d.
Crude extracts	0.97	108	49	n.d.	n.d.
Chlorosomes	<0.50	140	40	1.5	n.d.
Cytoplasmic membranes	2.00	54	114	3.7	n.d.

[a] Succinate dehydrogenase in *Chloroflexus* fractions was assayed without PMS, in *Chlorobium* extracts + PMS (phenazine methosulphate).
[b] n.d. = not detected

ponents varied (Table 3). As shown by Halfen et al. (1972) the main carotenoids of *Chloroflexus* are γ-carotene and its derivatives as well as β-carotene. Apparently the hydroxy derivative of γ-carotene is mainly converted into its glucoside.

Thus, differences in carotenoid composition in fractions from *Chloroflexus* were restricted to the amounts of free hydrocarbons and the glucosidic compound. The data in Table 3 clearly indicate that chlorosomes contained very little glucosidic γ-carotene. Most of the carotenoids present were γ- and β-carotene. The cytoplasmic membrane, in contrast, showed an increased amount of γ-carotene glucoside at the expense of γ-carotene. Also the β-carotene content was reduced in the cytoplasmic membrane.

A similar pattern was found in the fractions isolated from *Chlorobium* 6230. The main carotenoid of green photosynthetic sulfur bacteria is chlorobactene (Liaaen-Jensen et al., 1964), the aromatic derivative of γ-carotene. Its hydroxy-compound is also found in limited amounts (Schmidt and Schiburr, 1970). In isolated cytoplasmic membranes of this organism, however, the hydroxy-compound of chlorobactene became enriched, and a high percentage of chlorobactene-glucoside could be detected. Chlorobactene-glucoside was identified in extracts of some brown photosynthetic sulfur bacteria (Schmidt, unpublished). The data presented in Table 3 indicate that chlorobactene-glucoside was a typical component of the cytoplasmic membrane although chlorobactene itself was the predominant carotenoid. In chlorosomes of *Chlorobium* 6230 hydroxy carotenoids were only present in traces and the chlorobactene content was slightly reduced. Instead neurosporene and lycopene, the precursors of this compound, were present in increased amounts.

Other Components. For further characterization of the isolated fractions from both strains the contents of cytochrome *c*, phospholipids and glycolipids were determined (Table 4).

In chlorosomes the content of cytochrome *c* was low. The small amounts detected were probably due to contaminations by membrane fragments. Cytoplasmic membranes were rich in cytochrome *c* as was expected. The distribution of glycolipids was about the same in both organisms. As was stated by Cruden and Stanier (1970) for *Chlorobium* strains the major part of this component was localized in the chlorosomes. The amount was about three times of that found in cytoplasmic membranes.

Surprisingly, the distribution of phospholipids was different in both species. In *Chlorobium* cytoplasmic membranes contained three times as much as the chlorosomes whereas in fractions of *Chloroflexus* the major part of the phospholipids was enriched with the chlorosomes.

In general the total lipid content was significantly higher in cells of *Chlorobium* than in cells of *Chloroflexus*.

Enzyme Activities. As further marker for the degree of purity and for the grade of fractionation of the cells the specific activities of NADH-oxidase and especially succinate dehydrogenase were assayed (Table 4). Succinate dehydrogenase was clearly enriched with the cytoplasmic membrane fraction from *Chloroflexus*. This enzyme, however, could not be detected in chlorosomes. Unfortunately, we were unable to measure any activity of this enzyme in our preparations of cytoplasmic membranes from *Chlorobium* 6230, although Cruden and Stanier (1970) found quite a considerable activity in the membranes of all the strains which they examined.

In agreement with Cruden and Stanier (1970) NADH-oxidase activities were distributed in all the fractions of both strains studied in the present work. There was a slight increase of activity in cytoplasmic membranes of *Chlorobium* and a somewhat higher activity in the isolated chlorosomes from *Chloroflexus*. During the breakage of cells this enzyme apparently is easily released from cytoplasmic membranes. The 140,000 × *g* supernatant also had a very high NADH-oxidase activity. The increased activity in the chlorosome fraction of *Chloroflexus* thus may have been caused by the release of enzyme particles from the membranes during sonification. The degree of NADH-oxidase activity in these fractions varied in different experiments and, therefore, can be used as an indicator for the grade of fractionation.

Discussion

There are many similarities in the cytology and composition of cell components of Chlorobiaceae and Chloroflexaceae (Pierson and Castenholz, 1974a, b, 1978; Kenyon and Gray, 1974; Mandel et al., 1971; Madigan and Brock, 1977). In the members of both families the basic light harvesting BChl c is housed in chlorosomes (Chlorobium vesicles), separate organelles, bounded by a special protein layer. These organelles are in close contact with the reaction center complexes localized in the cytoplasmic membrane (Olson et al., 1976b; Staehelin et al., 1978). But as has been shown by the data given in this study there are some differences in pigmentation and chemical composition of the isolated cell fractions, and therefore probably in structural arrangements and function also. This may be due to the different physiologies of the two types of organisms. Chlorobiaceae are obligate phototrophs, dependent on reduced sulfur compounds as hydrogen donor and on the presence of CO_2 in the medium. In contrast, Chloroflexaceae are facultative phototrophs, not dependent on reduced sulfur compounds and CO_2. These organisms also show a much wider range of adaptability in response to a variety of culture conditions such as light intensities, temperature, and oxygen tension.

During the preparation of chlorosomes and cytoplasmic membranes it was observed that the membranes of *Chlorobium* cells appeared to be more labile to the methods employed than those of *Chloroflexus*. In all centrifugation steps the broken cells of *Chlorobium* 6230 released a considerable part of the BChl-components into the supernatants which exhibited peaks at ca 810 and 674 nm, just like the isolated cytoplasmic membranes. This indicates that these particles were released from this fraction. The corresponding supernatants from *Chloroflexus* fragments were rich in proteins and carotenoids only (ca. 50% as γ-carotene glucoside) but never showed remarkable amounts of BChl a and c. These differences in pigmentation of the supernatants may account for differences in construction of the cytoplasmic membranes of both strains. In addition it was observed that the cytoplasmic membranes from *Chlorobium* were always isolated in the form of vesicles (Fig. 3B) whereas cytoplasmic membranes of *Chloroflexus* accumulated mainly as flat pieses (Fig. 3A).

The kind of attachment of chlorosomes to the cytoplasmic membrane may be different too. In thin sections of whole cells of *Chloroflexus* a black layer (lipid?) at the site of attachment can be clearly distinguished (Pierson and Castenholz, 1974a, Staehelin et al., 1978). This layer obviously purifies with the chlorosomes during the isolation procedure causing the high phospholipid content of chlorosomes from *Chloroflexus*. From the analytical data it seems that this component stays with the cytoplasmic membrane in preparations of fragments of *Chlorobium* (Table 4). *Chloroflexus*-chlorosomes are attached to the membrane more tightly than those of *Chlorobium*. In electron micrographs of isolated and purified cytoplasmic membranes from *Chloroflexus* a few chlorosome-like structures were always present. On membranes isolated from cells with highly reduced BChl c-content (high light intensities, low temperature) these "incomplete" chlorosome structures were considerably enriched (Schmidt and Mayer, 1979) suggesting that only chlorosomes filled with Bchl c can easily be separated by mechanical disruption.

The absorption spectra of the isolated fractions revealed some important differences (Figs. 5 and 6). In purified chlorosomes Bchl c is the typical chlorophyll component. But in addition a small amount of Bchl a is present in chlorosomes of both strains. In chlorosomes of *Chloroflexus* this Bchl a-complex causes a shoulder at 790 nm and thus seems to be a Bchl a-complex characteristic of chlorosomes of this organism. It might attribute to the chlorophyll-protein which links the chlorosomes to the membranes and to its reaction center arrangement as has been claimed by Olson et al. (1976b) for *Chlorobium limicola*. The 790 nm-Bchl a, however, could not be identified from the spectra of chlorosomes from *Chlorobium* 6230. Probably the corresponding inflection is covered by the intense peak of Bchl c at 754 nm.

The spectra of the Bchl a-region of purified cytoplasmic membranes differed considerably in both strains. The absorption maxima at 806 and 868 nm in membranes of *Chloroflexus* resemble that of B800 and B850 chlorophylls of Rhodospirillaceae and Chromatiaceae (Thornber, 1978), suggesting that the photosynthetic unit of the cytoplasmic membrane of

Chloroflexus is different from that described for *Chlorobium limicola* by Olson et al. (1976a, b).

Concerning the specific carotenoid content and composition of individual carotenoids in the isolated fractions of both organisms there are some parallel tendencies. Chlorosomes, in general, contain more carotenoid than cytoplasmic membranes. In chlorosomes from *Chlorobium* 6230, however, the specific carotenoid content is much higher than that in the cytoplasmic membrane in comparison with the differences in carotenoid content of the corresponding fractions from *Chloroflexus* Ok-70-fl. The distribution of the individual carotenoids in chlorosomes and membranes revealed the same pattern in both species. Non-hydroxylated components were mainly localized in chlorosomes, the bulk of glucosidic carotenoids was found in cytoplasmic membranes. This fact indicates a differentiation of carotenoid biosynthesis in the different cell components and a different function for the individual carotenoid compounds at their sites of location. This suggestion is consistent with the findings that cytoplasmic membranes from *Chloroflexus* have an increased content of glucosidic OH-γ-carotene and its oxo-derivatives when cells are grown in stress conditions such as low temperature (Schmidt, unpublished) and high oxygen tension (Schmidt, 1976).

The distribution of all other components assayed (glycolipids, cytochrome *c*, enzyme activities) is about the same in fractions of both organisms. This indicates that, at least, both types of cytological structures bear the same basic photosynthetic functions in Chlorobiaceae as well as in Chloroflexaceae. But it seems that there are some differences in structural organization and pigment-protein-complexes, especially with respect to the organization of that part of the photosynthetic apparatus which is localized in the cytoplasmic membrane.

A study on the constitution and development of chlorosomes in connection with different growth conditions is in preparation. This will probably give more information about the organization of the photosynthetic apparatus and the regulation of photopigment synthesis in *Chloroflexus*.

Acknowledgement. I am grateful to Miss Margret Maarzahl for skillfull technical assistance, to Prof. Dr. F. Mayer for advice and help during the electron microscopic work and to Prof. Dr. R. W. Castenholz and Dr. R. J. Cogdell for reading the manuscript.

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Received October 18, 1979