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CHLOROPHYLL-PROTEIN COMPLEXES

VARIABILITY OF CPI, AND THE EXISTENCE OF TWO DISTINCT FORMS OF LHCP AND ONE LOW-MOLECULAR-WEIGHT CHLOROPHYLL *a* PROTEIN

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The thylakoids of chloroplasts from autotrophically cultivated *Chlamydobotrys stellata* contain six chlorophyll proteins: three chlorophyll *a*-protein complexes CPIa, CPIb and CPIc (155, 140 and 135 kDa), two forms of light-harvesting chlorophyll protein, LHCPa and LHCPb (36 and 32 kDa), and one chlorophyll *a* protein, CPIII, of low molecular weight (9 kDa). A change to photoheterotrophic nutrition by addition of acetate leads to a drastic alteration of the thylakoid arrangement in *Chlamydobotrys* chloroplasts: (1) The amount of CPIa and CPIc increases in relation to CPIb. (2) The amount of LHCPa is also raised, whereas that of KHCPb is decreased. LHCPb contains chlorophyll *a* and chlorophyll *b*. No chlorophyll *b* is detectable in LHCPa. (3) A new CPIV with chlorophyll *a* only was identified (100 kDa). These modifications of the photosynthetic apparatus are discussed in connection with the known requirements of photoheterotrophically cultivated *C. stellata* for Photosystem I activity.

Introduction

The green volvocean alga *Chlamydobotrys stellata* has the unique ability to change the ultrastructure of its chloroplasts and its photosynthetic activity according to its nutritional conditions. In the case of autotrophic growth, requiring complete photosynthetic electron transport, the organisms do not differ from other unicellular green algae. The replacement of CO_2 by acetate, however, which requires only Photoststem I activity for photoassimilation of the carbon source [1,2], leads to almost granafree chloroplasts [3,4], changes in Photosystem II activity [5,6] and decreases the Photosystem II-dependent electron transport to Photosystem I [7]. These ultrastructural and physiological differences between autotrophically and photoheterotrophically cultivated *C. stellata* are accompanied by alterations of the forms of native chlorophyll [8,9]. Curve analyses of the absorption spectra [9] at liquid N_2 temperature suggest an increase of the longer wavelength forms of chlorophyll *a* (λ 685,693 and 707 nm) in the cells grown in presence of acetate. It was proposed [9] that these results are induced by the requirement of *C. stellata* for, respectively, Photosystem I and Photosystem II activities and indicate alterations of the functional chlorophyll proteins. This makes *C. stellate* a most suitable organism in studies on the possibility of

Abbreviations: CP, chlorophyll-protein complex; Deriphat 160, sodium-*N*-lauryl-β-iminodipropionate; LHCP, light-harvesting chlorophyll-protein complex; SDS, sodium dodecyl sulfate,; Tricine, *N*-tris(hydroxymethyl)methylglycine; kDa, kilodalton.

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variation in plastidial proteins in fully green algae without mutation or unphysiological treatment of the organisms.

Polyacrylamide gel electrophoresis with sodium dodecyl sulfate for the separation of plastid proteins could not be applied until now to *C. stellata* because of the high amounts of free chlorophyll liberated during electrophoresis. This made quantitative statements as to the thylakoidal contents of the various chlorophyll proteins impossible. In this investigation we therefore report on chlorophyll proteins from autotrophically and from photoheterotrophically cultivated *C. stellata* separated by polyacrylamide gel electrophoresis with Deriphat 160 [10] at 4°C, which eliminates the occurrence of free chlorophyll. Evidence will be presented on drastic quantitative and qualitative changes of high-molecular-weight chlorophyll protein induced by nutrition of the algae and on the occurrence of high amounts of a 9 kDa chlorophyll protein.

Materials and Methods

Chlamydobotrys stellata, strain 10-1e (Sammlung von Algenkulturen, Pflanzenphysiologisches Institut, Universität Göttingen), was cultivated in continuous light either autotrophically with CO_2 or heterotrophically with acetate as carbon source [11]. For preparation of thylakoids, cells were harvested from exponentially grown cultures and broken in a YEDA press (Research and Development Co., Ltd., Rehovot, Israel). Fragment of thylakoids were isolated on a Ficoll/saccharose/glycerol gradient [12], resuspended in a medium comprising 0.33 M sorbitol/1 mM $MgCl_2$/2 mM EDTA/4 mM mercaptoethanol/50 mM Tricine-KOH, pH 8.4 [12]. To fractionate the chlorophyll proteins, the washed membrane preparation was incubated with 0.1% sodium deoxycholate (0.1% sodium deoxycholate/0.25 M saccharose/10 mM Tris-HCl (pH 8.0)/1 M KCl) for 40 min at 4°C. This membrane suspension was centrifuged for 1 h at 100 000 × *g*. The resulting supernatant is the first membrane fraction. The pellet was incubated once more with sodium deoxycholate (0.75% sodium deoxycholate/0.5 M saccharose/34 mM Tris-HCl (pH 8.0)/1 M KCl) for 40 min at 4°C and subsequently centrifuged for 20 min at 100 000 × *g*. The resulting supernatant is the second membrane fraction and the resulting pellet is the third membrane fraction [12]. The first and the second membrane fractions contain extrinsic membrane proteins [12], but they do not contain chlorophyll proteins. Thus, only the third membrane fraction was used in this investigation. Its plastidial membrane proteins were separated without heat denaturation in 15% acrylamide gels [12] containing 1% Deriphat 160 [10].

The estimation of the relative chlorophyll a contents of the chlorophyll proteins was derived from scanning unstained gels at 675 nm on a Quick Scan Jr TCL (Helena Lab., USA). Absorption spectra in the spectral region between 400 and 750 nm (Shimadzu spectrophotometer UV-200, Shimadzu Seisakusko Ltd., Kyoto, Japan) of the chlorophyll proteins were recorded directly in slab-gel segments without elution. For pigment analysis, the protein solutions were extracted with methanol. The chlorophyll *a*/chlorophyll *b* ratio was determined according to Ogawa and Shibata [13].

Results

Thylakoids of chloroplasts from autotrophically cultivated *C. stellata* contain six chlorophyll proteins (Fig. 1): three chlorophyll *a* protein complexes with molecular weights of 155, 140 and 130 kDa, respec-

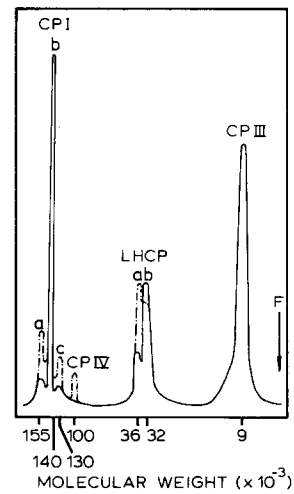

Fig. 1. Distribution of chlorophyll between the chlorophyll-protein complexes resolves by acrylamide-gel electrophoresis of thylakoids of *Chlamydobotrys stellata*. ———, autotropic cells; · – · – ·, photoheterotrophic cells; F = front.

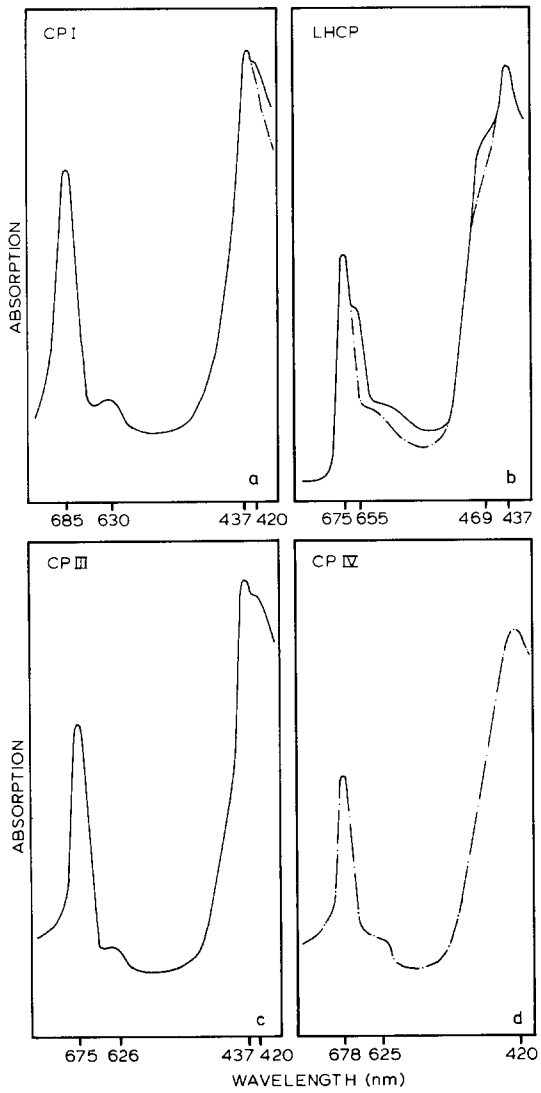

Fig. 2. Absorption spectra of the chlorophyll-protein complexes separated by polyacrylamide gel electrophoresis of thylakoids of *C. stellata*. ———, autotrophic cells, · – · – ·, photoheterotrophic cells.

tively (CPIa, CPIb and CPIc), two chlorophyll proteins with molecular weights of 36 and 32 kDa, respectively (LHCPa and LHCPb) and one chlorophyll *a* protein with a molecular weight of 9 kDa (CPIII). The chlorophyll proteins CPIb and CPIII account for most of the chlorophyll, whereas the others are only minor components. Colored material did not remain at the top of the gels. Under our conditions the amount of free chlorophyll generated by electrophoresis was none or negligible, whereas degenerating conditions such as SDS-treatment or electrophoresis without sufficient cooling liberate chlorophyll migrating in the front together with bromophenol blue. CPIII is distinct behind this front. Evidence for a protein complexed with chlorophyll is given by staining with Coomassie blue [12] or by highly sensitive silver staining after extraction of lipids and reelectrophoresis of the isolated CPIII.

The absorption spectrum of CPI (Fig. 2a) is characterized by maxima at 685 and 437 nm, and a shoulder at 420 nm. The absorption maximum of CPIa is at 680 nm, of CPIb at 685 nm and of CPIc at 687 nm (Fig. 3). This extremely high values are in agreement with other investigations on absorption spectra of *C. stellata* [9]. The complex LHCP contains chlorophyll *a* and *b*. The absorption spectrum has maxima at 675 and 437 nm and two shoulders at

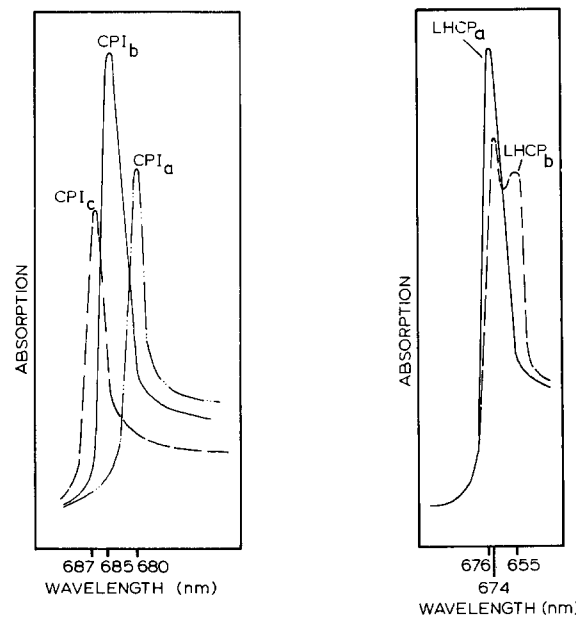

Fig. 3. (Left.) Absorption spectra of the chlorophyll-protein complexes CPIa (· – · – ·, CPIb (———) and CPIc (– – –) isolated by polyacrylamide gel electrophoresis from thylakoids of *C. stellata*.

Fig. 4. (Right.) Absorption spectra of the chlorophyll-protein complexes LHCPa (———) and LHCPb (– – –) isolated by polyacrylamide gel electrophoresis from thylakoids of *C. stellata*.

655 and 469 nm (Fig. 2b). Two components of LHCP can be separated and distinguished from each other by their absorption spectra (Fig. 4): LHCPa contains only chlorophyll *a* (absorption maximum at 676 nm) and LHCPb chlorophyll *a* and *b* in a ratio 1.4 : 1 (absorption maxima at 674 and 655 nm, respectively).

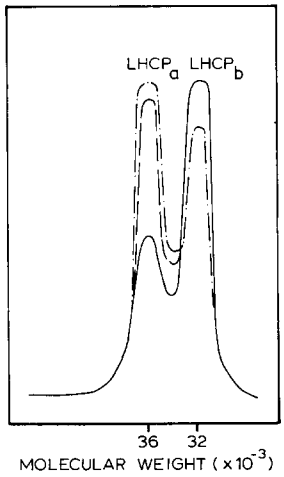

Fig. 5. Distribution of chlorophyll between the chlorophyll-protein complexes LHCPa and LHCPb isolated from *C. stellata* at various times during adaptation from autotrophic to photoheterotrophic growth. ———, autotrophic growth; – – –, 1.5 h photoheterotrophic growth, · – · – ·, 6 h photoheterotrophic growth.

The low-molecular-weight complex CPIII contains only chlorophyll *a*. Its absorption spectrum has maxima at 675 and 437 nm (Fig. 2c) and is, therefore, unlike the absorption maxima of liberated chlorophyll at a wavelength lower than 670 nm.

Photoheterotrophic nutrition of *C. stellata* affects the thylakoidal chlorophyll proteins in several ways:
(a) The amounts of CPIa and CPIc are increased in relation to CPIb (Fig. 1) and the absorption spectrum of CPI has lost the weak shoulder at 420 nm (Fig. 2b), perhaps caused by missing a carotenoid.
(b) The amount of LHCPa is also raised (Fig. 1), whereas that of LHCPb is decreased. With a decrease in LHCPb the chlorophyll *b* content of the whole LHCP is lowered. The shoulders of the absorption spectrum at 655 and 469 nm are no longer detectable.
(c) A new protein-complex can be identified with a molecular weight of 100 000 containing only chlorophyll *a* (CPIV, Fig. 1). Its absorption spectrum resembles that of CPI or LHCPa (Fig. 2d).

During the adaptation of *C. stellata* from autotrophic to photoheterotrophic nutrition, the amount of LHCPa rises slowly during the first 6 h of the adaptation process (Fig. 5). This rise is paralleled by a decrease in chlorophyll *b* content of LHCP (Table I). After 6 h a final chlorophyll *a*/*b* ratio of 8.5 : 1 is reached. In addition, the ratio of CPI to LHCP is also altered (Fig. 5). After the 12th hour of adaptation a ratio of 2 : 1 can be measured (Table II). Because of the low amount of CPIV, the change of this thylakoid protein could not be followed with accuracy in the same way.

TABLE I

CHLOROPHYLL *a*/CHLOROPHYLL *b* RATIO OF THE $LHCP_a + LHCP_b$ ISOLATED FROM *C. STELLATA* AT VARIOUS TIMES DURING ADAPTATION FROM AUTOTROPHIC TO PHOTOHETEROTROPHIC GROWTH

Time of photoheterotrophic growth (h)	Chlorophyll *a*/chlorophyll *b*
0	1.4 (±0.3)
1.5	2.3 (±0.4)
3.5	7.0 (±0.7)
6	8.4 (±0.3)
12	9.0 (±0.2)

TABLE II

QUOTIENTS OF CHLOROPHYLL-PROTEIN COMPLEXES ISOLATED FROM *C. STELLATA* AT VARIOUS TIMES DURING ADAPTATION FROM AUTOTROPHIC TO PHOTOHETEROTROPHIC GROWTH

Time of photoheterotrophic growth (h)	Ratio	
	LHCPa/LHCPb	CPI/LHCP
0	0.46 (±0.08)	1.22 (±0.09)
1.5	1.04 (±0.23)	0.92 (±0.02)
3.5	1.31 (±0.01)	1.13 (±0.17)
6	1.68 (±0.24)	1.17 (±0.08)
12	1.77 (±0.15)	2.15 (±0.18)

Discussion

The data from this investigation demonstrate the existence of three major and several quantitatively minor chlorophyll-protein complexes in *C. stellata.*

Of the high molecular weight chlorophyll proteins, the complex CPIb is qualitatively similar to the ubiquitous *P*-700-chlorophyll *a* protein described in the literature [14]. The extremely high value of the absorption maximum is a characteristic property of *C. stellata* [9]. The component CPIa might be a supramolecular complex that contains CPI as its principal constituent [15]. Additional chlorophyll *a* proteins of high molecular weight detectable in the neighborhood of CPIb, such as CPIc, have also been found by others [16,17]. It is not yet known what is lost from CPI to cause it to migrate more rapidly: either an electron-acceptor species and/or antenna chlorophylls and/or a part of the reaction center. The components CPIa and CPIc are especially subjects of quantitative changes due to nutritional conditions. Their concentrations rise in photoheterotrophically grown algae from 5.8 to about 15.3% of the total chlorophyll content of CPI, compared to autotrophic cells, for CPIa and from 4.8 to 8.4% for CPIc. The quantitative relationship between the concentrations of these two complexes and the known requirement for high Photoststem I activity in the acetate-grown cells of *C. stellata* [1,2,18] lead to the hypothesis that the increase in CPIa and CPIc is directly related to the requirements for high Photosystem I activity.

CPIV is possibly identical with a chlorophyll *a* protein or perhaps an oligomer of it resolved between CPI and LHCP in higher plants and other algae [15–17]. It is suggested to be an oligomer of LHCPa [15].

The LHCP complex is qualitatively identical to the light-harvesting chlorophyll *a/b* protein [14]. When the thylakoids have been prepared from autotrophic cells, the chlorophyll *a/b* ratio of this complex of about 1.4 is in agreement with the values reported by others [15,19–21]. Our observations, however, contradict the equimolar quantities of chlorophyll *a* and *b* reported by others [22,23]. This complex is made of two chlorophyll proteins distinguishable from each other by their chlorophyll *a/b* ratios. Because one of the two is increased under nutritional conditions requiring only Photosystem I for photoassimilation, the assumption of only one common light-harvesting complex must be questioned. It has to be mentioned, in addition, that the LHCPa content of the thylakoids increases parallel to the loss of stacking [3,4,24].

The amount of CPIII is less subject to nutrition of algae. Its cytological and functional role is uncertain, even though low molecular-weight chlorophyll proteins have been observed several times [12,25]. Lagoutte and Duranton [26] have demonstrated a structural protein of a size of 10 kDa, which after their assumption is basic to all chlorophyll-protein complexes of the thylakoids.

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