Chlorophyll-Protein Complexes of *Chlorella fusca*

Aloysius Wild and Barbara Urschel

Universität Mainz, Institut für Allgemeine Botanik, Saarstr. 21, D-6500 Mainz

Z. Naturforsch. **35 c,** 627–637 (1980); received March 19, 1980

Chlorella, Chlorophyll-Protein Complexes, Polyacrylamide-Gel Electrophoresis, Standard Curves for Characterization of Chlorophyll Proteins

Chlorophyll-protein complexes from thylakoids of the normal type and two mutants of *Chlorella fusca* were separated using sodium dodecyl sulfate acrylamide gel electrophoresis (PAGE). The properties of the chlorophyll-protein complexes of the three strains of *Chlorella* were compared. Standard curves were set up for the characterization of the chlorophyll-proteins. In every electrophoretic separation of chlorophyll-protein complexes, a certain amount of pigment is separated from the protein. We tried to keep that amount as low as possible by mild solubilization and by working in low temperature. Under these conditions, we obtained several new chlorophyll-proteins in addition to the P-700-chlorophyll a-protein (CP I) and the light-harvesting chlorophyll a/b-protein (CP II).

Thus, a small band (CP Ia) was located between the top of the gel and the CP I after electrophoresis. Although it shows spectral qualities similar to CP I, it possesses a much lower chlorophyll a/chlorophyll b ratio. It may be an aggregate of photosystem I and light-harvesting chlorophyll.

We found and analyzed three other chlorophyll-proteins with electrophoretic mobilities intermediate between that of the P-700-chlorophyll a-protein and that of the light-harvesting chlorophyll a/b-protein complex. Two of these chlorophyll-proteins, the $LHCP^1$ and the $LHCP^2$, have a low chlorophyll a/chlorophyll b ratio and spectral properties similar to that of the light-harvesting chlorophyll a/b-protein ($LHCP^3$). They obviously represent dimers or oligomers of the latter complex.

A third, new complex (CPa) migrated between $LHCP^3$ and its dimer. With the chlorophyll b deficient mutant G 36 of *Chlorella fusca,* this complex could be obtained in high purity and great enrichment (15% of total chlorophyll). It could be proved that this complex only contains chlorophyll a. Its red absorption maximum is at 671 nm. Some indirect evidences suggest that it may be a good candidate for the PS II reaction center complex.

Introduction

Since substantial evidence of two chlorophyll-protein complexes in higher plants was established by solubilization of the thylakoid membranes with an anionic detergent and a following polyacrylamide-gel electrophoresis [1, 2] an ever increasing amount of data on this class of proteins has accumulated. Three chlorophyll-containing bands were obtained in electrophoresis. Whereas the zones of the lowest and intermediate electrophoretic mobility represent chlorophyll-protein complexes, which were at first called complex I (CP I) and complex II (CP II), the third zone migrating at the front consists of pigments and lipids complexed to detergent, termed the "free pigment" [2, 3].

As complex I is highly enriched in P-700 (Chl/P-700 = 40 [4]; Chl/P-700 < 20 [5]) and contains the reaction-center of PS I, it was called P-700-Chl a-protein [6]. The complex, which should only contain Chl a [7] is ubiquitous in the plant kingdom [7, 8]. The complex represents 4–30% of the total Chl in all organisms examined [8] with 10–18% being typical of its content in higher plants [4, 7].

Additional CP I bands, showing a high Chl a/Chl b ratio, were described recently [9–11].

Since higher plants can live photosynthetically in the complete absence of complex II ($LHCP^3$) its obvious functions are to absorb light energy and to transfer this energy preferentially to the reaction-center of PS II. Therefore this complex is named light-harvesting chlorophyll a/b-protein [12]. It probably occurs in all Chl b-containing plants; it is said to comprise the entire amount of Chl b of plants and is said to possess equimolar quantities of Chl a and

Abbreviations: A, absorbance; Chl, chlorophyll; CP I, P-700-chlorophyll a-protein; CP II, $LHCP^3$, light harvesting chlorophyll a/b-protein; F, area; FC, free chlorophyll; LDW, growth conditions: 16 h light/8 h dark; LH, light harvesting; NF, normal strain; PAGE, polyacrylamide gel electrophoresis; PS, photosystem; SDS, sodium dodecyl sulfate.

Reprint requests to Prof. Dr. A. Wild.

0341-0382/80/0700-0627 $ 01.00/0

Chl b [7, 13]. Its proportion of the total chlorophyll accounts for 40–60% in higher plants and green algae [7, 12, 14].

It is likely that aggregates of LH-monomers exist *in vivo* [15] and dimers and oligomers ($LHCP^1$, $LHCP^2$) were actually found in detergent extracts several times. This assumption is mainly based on agreement of the absorption spectra [9–11, 16–22].

Other chlorophyll-protein complexes, which have not been characterized so far, could sometimes be observed [8, 17, 23].

For a long time, it seemed to be impossible to isolate by PAGE the PS II reaction center antennae complex, as it is very unstable [3]. Recently, however, a complex was reported to migrate between $LHCP^3$ and its dimer in electrophoresis. This complex may represent the PS II reaction center antennae [9–11, 20, 22, 24]. It is supposed to contain Chl a only.

Most data on chlorophyll-protein complexes that have been published so far are related to higher plants. This report intends to give information on the Chl-protein complexes which were obtained from two mutants and the normal type of *Chlorella fusca* after solubilization with SDS and PAGE at 2 °C.

The characterization of the complexes such as determination of the Chl a/Chl b ratio and the determination of the relative proportions of the complexes was achieved by elution of the bands as well as by standard curves. The naming of the complexes is according to Anderson *et al.* [10].

Materials and Methods

Plants

The mutants G 36 (C. 1.1.10.36) and G 10 (C. 1.1.10.10) of *Chlorella fusca* [25] and the normal strain were examined. In the chlorophyll b-deficient mutant G 36, the thylakoid membranes are in a primary molecular organization stage, characterized by small photosynthetic units, deficient grana stacking of thylakoids and deficiency of antennae chlorophyll, especially of Chl b [26–29]. The mutant G 10 differs from the wild type that it is not able to synthesize Chl in the dark. Exposure of dark-grown etiolated cells of the mutant to light initiates the greening process and results in the formation of full photosynthetic capacity of chloroplasts [30].

Growth conditions

The cells were grown under following conditions: at 24 °C, on a shaker in liquid medium supplemented with glucose (0.2%), under low light conditions (about 2000 lux), in a 16 h light/8 h dark schedule. In order to attain different greening stages of the mutant G 10, cells were grown in the dark till the beginning of light exposure. The cultivation of the mutant G 10 lasted six days, that of the normal type and the mutant G 36 seven days. Mutant G 36 was exposed to diffuse light in the first two days. The medium and culturing conditions were described previously [28, 31, 32].

Isolation of thylakoids

Cell homogenates were prepared from 200 ml algae suspension. After centrifugation, the pelleted cells were resuspended in 15 ml of a buffer solution consisting of 0.05 mol · l^{-1} tricine-KOH (pH 8.0); 0.075 mol · l^{-1} KCl; 0.35 mol · l^{-1} mannitol. Homogenization was carried out according to Wild and Fuldner [29]. The broken-cell suspension was centrifugated ($5000 \times g$, 10 min) and the pellet was resuspended in a medium containing 0.05 mol · l^{-1} Tris-borate; 0.002 mol · l^{-1} $MgCl_2$ (pH 8.2). In order to remove soluble protein, the pellet was washed twice with this medium. Then a suspension with 0.5 mg Chl · ml^{-1} was prepared with this medium. After addition of SDS (from a 10% w/v stock solution) – according to the SDS/Chl ratio – the suspension was sonicated in order to attain a better solubilization (15 · 15 sec, 25 W, Branson Sonifier B-12, micro tip special). For the separation of the insoluble components, a 10-minute centrifugation at $26000 \times g$ was made. The supernatant was used for the electrophoretical separation. All the methods described above were carried out at 4 °C and in the dark. The SDS-incubation period was 30 min. The SDS/Chl ratio differed with the three Chlorella strains: mutant G 36, SDS/Chl = 80 (w/w); mutant G 10, SDS/Chl = 10; normal strain, SDS:Chl = 10 and 40.

PAGE

Chlorophyll-protein complexes were separated by polyacrylamide gel electrophoresis (PAGE) using as running buffer 0.05 mol · l^{-1} Tris-borate pH 8.2, which contained 0.1% SDS (w/v). Gels were made in 0.6 diameter and 10 cm long glass tubes (filling height: 8 cm). They consisted of 8% acrylamide and

0.21% (w/v) N,N′-methylenebisacrylamide made up in 0.375 mol · l^{-1} Tris-borate (pH 8.2) plus 0.1% (w/v) SDS and 0.05% (v/v) N,N,N′,N′-tetramethylenediamine (TEMED). The polymerization was started by ammonium persulphate (0.043% w/v). For each gel, 5–10 µl SDS extract was used. Electrophoresis was carried out at 3 mA/gel in the first 10 min, then at 6 mA/gel (corresponding 350 V). The whole electrophoresis lasted 30 min, which corresponds to the migration distance of 2 cm.

During electrophoresis, the apparatus was darkened and the gels were kept cool at 2 °C.

Gel scanning was obtained in situ on the gel, using the gel scanning attachment of a Zeiss PMQ II spectrophotometer. The scanning was carried out at 653 and 672 nm.

Isolation and characterization of chlorophyll-protein complexes

The corresponding gel bands of 8–10 gel columns were cut out and extruded through a nylon mesh with pores of 0.5 mm^2. The diffusion of the chlorophyll-protein complexes from the gel into the buffer, containing 0.05 mol · l^{-1} Tris-HCl (pH 8.2) and 0.02% SDS (w/v) took place in the dark at 0 °C and lasted 12 h. After centrifugation of the gel particles at 5000 × *g* (30 min), absorption spectra were recorded by an Aminco DW-2 UV-VIS-spectrophotometer. The recording of absorption spectra was repeated after dilution of the buffer solution with acetone (1:4). The spectra of these acetonic solutions were used to measure the proportion of the complexes in the total amount of chlorophyll as well as the Chl a/Chl b ratio according to Ziegler and Egle [33].

The direct quantitative analysis of the densitometric tracings was possible by means of standard curves for chlorophyll and for the Chl a/Chl b ratio [34]. The estimation of the relative Chl proportions of the various chlorophyll-protein complexes was derived from the 672 nm scans, using a planimeter to measure the area under each peak. Each value is the average of 6 different scans. The content of chlorophyll or rather the content of Chl a of the various bands, estimated by elution, could be related to corresponding peaks in densitometric tracings. The plotting of the Chl a + b content against the areas of the corresponding peaks (F_{672}) yields a standard curve for the estimation of total Chl. Corresponding to this, a standard curve for the determination of Chl a was obtained by plotting the Chl a content against the area under the corresponding peak F_{672}.

For the setting-up of a standard curve for the Chl a/Chl b ratio, the absorbance at 653 and 672 nm was attained from the absorption spectra. The quotient A_{653}/A_{672} was plotted against the Chl b/Chl a ratio, which was ascertained from the acetonic solution of the same sample. For the analysis of the densitometric tracings, the absorbance at the peak maximum was measured and then the quotient A_{653}/A_{672} for each band was formed.

Results

Chl content and Chl a/Chl b ratio of the cells

The results reported and summarized in Table I show that the low chlorophyll content of the yellow mutant G 36 is combined with a very high Chl a/Chl b ratio.

Compared to the normal type C. 1.1.10, the normally greened mutant G 10 has a high Chl content but also a high Chl a/Chl b ratio. The value of the Chl a/Chl b ratio lies between that of the normal type and the mutant G 36. With different illumination periods of dark grown mutant G 10 cultures, each greening stage can be attained. Thus a short illumination period results in a decrease of chlorophyll and an increase of Chl a/Chl b ratio [30].

Table I. Chl a/Chl b ratio, chlorophyll content and dry weight of the normal strain and the mutants of *Chlorella fusca*.

Plant material	Chl a / Chl b	mg Chl / g dry weight	mg dry weight / l suspension
normal strain	2.46	24.1	1.04
mutant G 36	6.30	5.6	0.67
mutant G 10 (LDW)	3.52	32.0	0.81
mutant G 10 (6 h light)	4.34	3.5	0.73

The determination of standard curves for the measurements of chlorophyll from the scans

For rapid determination of chlorophyll and of the Chl a/Chl b ratios from the scans, standard curves were made for Chl a (Fig. 1), for total chlorophyll and for the Chl b/Chl a quotient (Fig. 2). For the estimation of Chl, the correlation coefficient of the standard curve is 0.92, and for that of Chl a it is 0.93. The inverse of the corresponding standard curve, with a correlation coefficient of 0.93, was used for measuring the Chl b/Chl a ratio.

Chlorophyll-protein complexes of the mutant G 36

As SDS strips off chlorophyll from the complexes [1, 7, 35], the solubilization was carried out carefully. Due to its low Chl content, mutant G 36 can only be solubilized with difficulty. The lowest SDS/Chl ratio must be found out to dissolve lamellae fully. A good electrophoretical separation of this yellow mutant was only possible at SDS/Chl = 80. Under these conditions no colored material remained at the top of the gel. Fig. 3 shows the scans at 672 and 653 nm of a representative gel pattern. In addition to the three generally known bands (the P-700-chlorophyll a-protein, the light-harvesting chlorophyll a/b-protein, and the free pigment zone), two other bands turned up in the gel: a small band (LHCP[1]) showing the same absorption spectrum as LHCP[3] and obviously an oligomer of LHCP[3], and another distinct zone (CPa), which migrated to a position between the LHCP[3] and its oligomer.

The new complex is of a bluish-green color. A comparison of the scans at 672 and 653 nm reveals a strong reduction of the peak on the 653 nm scan (Fig. 3). Because of the enrichment of Chl a in this complex, it should be termed CPa. The high Chl a content is reflected in the absorption spectrum of CPa, having two maxima at 671 nm and 437 nm (Fig. 4). Although the spectrum is identical in both maxima with that of LHCP[3], it lacks the shoulders at 653 and 470 nm usually caused by Chl b. The CPa complex is very sensitive to warmth.

The spectrum of LHCP[3] is quite similar to that described by Kan and Thornber [14] for the LHCP of the green alga *Chlamydomonas reinhardii.*

The P-700-Chl a-protein complex is characterized by maxima at 676 and 437 nm. The lack of shoulders at 653 nm and nearly 470 nm shows the absence of Chl b.

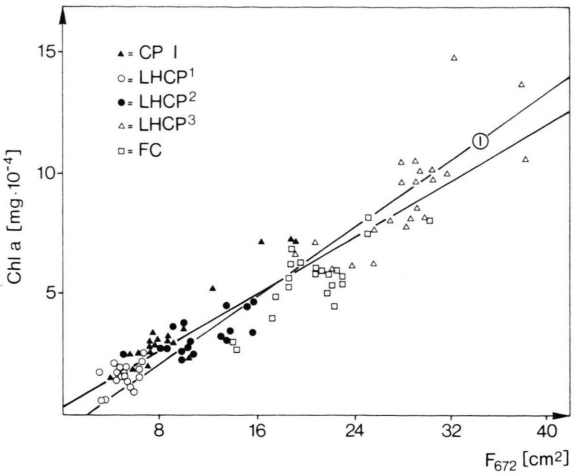

Fig. 1. Standard curve for the in situ determination of Chl a from the scans of the gel bands at $\lambda = 672$ nm. Plotting the Chl a contents against the areas of the corresponding peaks (F_{672}) yields the standard curve. Equation for the regression line: $y = 2.9 \cdot 10^{-5}\, x + 3.1 \cdot 10^{-5}$ (value of the correlation coefficient = 0.93) and for the inverse line I:

$$y = \frac{x - 1.65 \times 10^{-4}}{2.91 \times 10^{-4}}$$

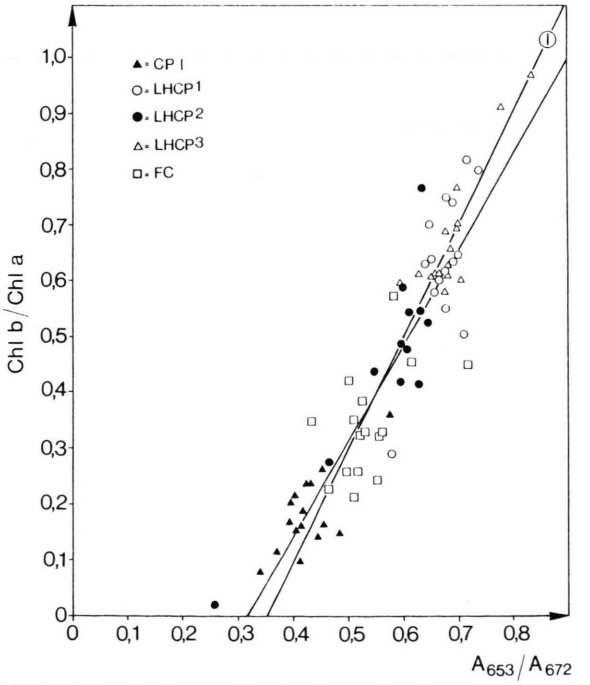

Fig. 2. Standard curve for the determination of the Chl a/Chl b ratio in situ from the densitometric tracings of the gel bands. Plotting the Chl b/Chl a ratios against the quotient A_{653}/A_{672} yields the standard curve. Equation for the regression line: $y = 1.71\, x - 0.54$ (value of the correlation coefficient = 0.92) and for the inverse line I:

$$y = \frac{x - 0.35}{0.5}$$

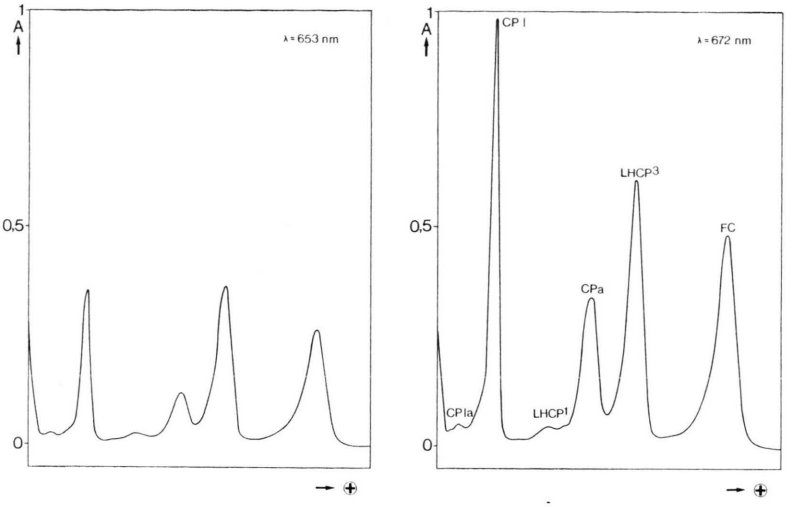

Fig. 3. Distribution of chlorophyll-protein complexes of the mutant G 36 by scanning unstained gels in situ at $\lambda = 672$ and $\lambda = 653$ nm. For solubilization of thylakoids the SDS/Chl ratio was 80.

The elution results, presented in Table II, suggest that CPa contains only Chl a (in detail, Chl a/Chl b values > 46 were found). This suggestion could distinctly be proved by means of the densitometric analysis with the standard curves. Elution makes a complete separation of CPa zones from premigrating LHCP[3] difficult. This problem can easily be eliminated by analyzing the scan with the aid of standard curves, as in that case only the peak maximum has to be measured. Table II illustrates that the CPa complex of mutant G 36 accounts for some 15% of the total of chlorophyll.

An exact determination of LHCP[1] data was impossible because of its small size.

Experiments with low SDS concentrations (SDS/Chl = 10; 20; 40) show an unsatisfactory solubilization and resolution. But under these conditions, additional bands appear; they are reduced with increasing SDS/Chl ratio. Thus two bands migrate behind CP I; moreover an additional band migrating between LHCP[1] and the CPa complex was observed. All these bands have an essentially lower Chl a/Chl b ratio than the migrating complexes in front. Higher SDS quantities led to a decline of Chl b.

Chlorophyll-protein complexes of the normal strain

In the electrophoresis of the normal type of *Chlorella fusca*, up to seven bands can be distinguished at low SDS/Chl ratios.

Between the starting line at the top of the gel and the P-700-Chl a-protein complex migrates a yellowish green band which has three peaks in the densitometric tracing. An increase of SDS/Chl ratios leads to a small reduction of this band.

At low SDS/Chl ratios two weak yellowish green bands can be recognized between the CP I and LHCP[3] complex. Their absorption spectrum is in keeping with that of the LHCP[3]: thus, they obviously represent oligomers of CP II (LHCP[1] and LHCP[2]). They totally vanish at SDS/Chl = 40.

The mobility of the CPa complex is very similar to that of the LHCP[3], which together with the large quantities of the LHCP[3] makes the resolution of the

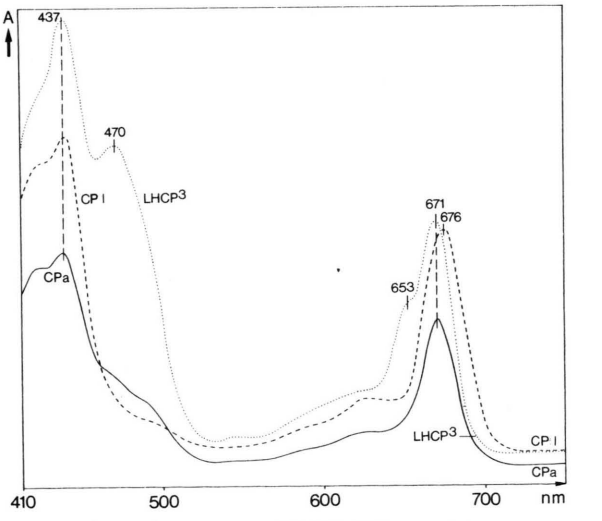

Fig. 4. Absorption spectra of P-700-Chl a-protein, CPa and LHCP[3] of the mutant G 36 after diffusion of the Chl-protein complexes from the gel in the Tris-HCl, SDS buffer. For solubilization of the thylakoids the SDS/Chl ratio was 80.

Table II. Relative proportion of Chl-protein complexes and the Chl a/Chl b ratios of the mutant G 36 according to different methods used for Chl determination.

Method		CP I	LHCP[1]	CPa	LHCP[3]	FC
elution	% Chl	23.8	2	15.5	34.5	24.2
	Chl a/Chl b	only Chl a	–	18.0	1.6	3.9
standard curves	% Chl	18.5	6.8	14.6	32.1	28.0
	Chl a/Chl b	only Chl a	1.6	only Chl a	1.9	3.7

Table III. Relative proportion of Chl-protein complexes and Chl a/Chl b ratios of the normal strain using different SDS/Chl ratios for solubilization of thylakoids and different methods for Chl determination.

SDS/Chl	method		CP Ia	CP I	LHCP[1]	LHCP[2]	CPa	LHCP[3]	FC
10:1	elution	% Chl		12.9		7.1		70.4	9.5
		Chl a/Chl b	2.8	4.9		1.8	2.4	1.3	4.1
10:1	standard	% Chl		12.1		5.2		64.5	18.4
	curves	Chl a/Chl b	2.5	18.5	1.8	1.9	2.8	1.3	4.4
40:1	standard	% Chl		11.7	–	–	6.0	58.3	23.7
	curves	Chl a/Chl b	2.8	only Chl a	–	–	7.0	1.3	2.7

Table IV. Relative proportion of Chl-protein complexes and Chl a/Chl b ratios of the greened mutant G 10 by means of standard curves for determination.

	CP I a	CP I	LHCP[1]	LHCP[2]	CPa	LHCP[3]	FC
%Chl	12.4		5.7	15.6		43.4	23.1
Chl a/Chl b	2.9	24.0	1.9	2.2	3.7	1.7	4.9

CPa complex difficult. At SDS/Chl = 10, only a weak shoulder at the starting end of the CP II peak can be recognized. From SDS/Chl ratio = 40 onwards, the densitometric tracing reveals a separated, distinct peak for the CPa-complex. Its area at 653 nm is strongly reduced compared with that at 672 nm (Fig. 5). This is due to the high Chl a content. The analysis of densitometric tracings by means of standard curves yields a Chl a/Chl b ratio of 7 (Table III). This value becomes essentially higher at a further raising of the SDS content.

The absorption spectrum of the P-700-Chl a-protein complex of the normal strain is characterized by maxima at 674, 437 and 422 nm.

The spectrum of the band CP Ia, which migrates between the top of the gel and CP I, shows great similarity to the absorption spectrum of P-700-Chl a-protein, due to its maximum at 673 nm. But the determination of the Chl a/Chl ratio by the densitometric tracings yields a value of 2.85. This value is higher than the corresponding value for LHCP but much lower than that for CP I. It was impossible to separate the two bands by means of elution.

The light-harvesting Chl a/b-protein complex and its oligomers show corresponding absorption spectra: They are marked by maxima at 670, 470, and 437 nm and a shoulder at 653 nm. Table III sums up the results of the elution and the analysis of the densitometric tracings of the normal form.

Chlorophyll-protein complexes of the mutant G 10

The electrophoresis of the normally greened mutant G 10 leads to band patterns corresponding to the normal type; but the mutant differs from the normal type in the strength of the individual bands (Fig. 6). Most striking is the strength of CP II-oligomers (LHCP[1] and LHCP[2]), whereas the peak of LHCP[3] is smaller than that of the normal strain.

This mutant also shows a yellowish green band which frequently produces several peaks in densitometric tracing and which migrates between the starting line at the top of the gel and CP I.

Between LHCP[3] and its dimer migrates the CPa complex. Concerning the mutant G 10, this zone is strongly overlapped by neighboring bands. This

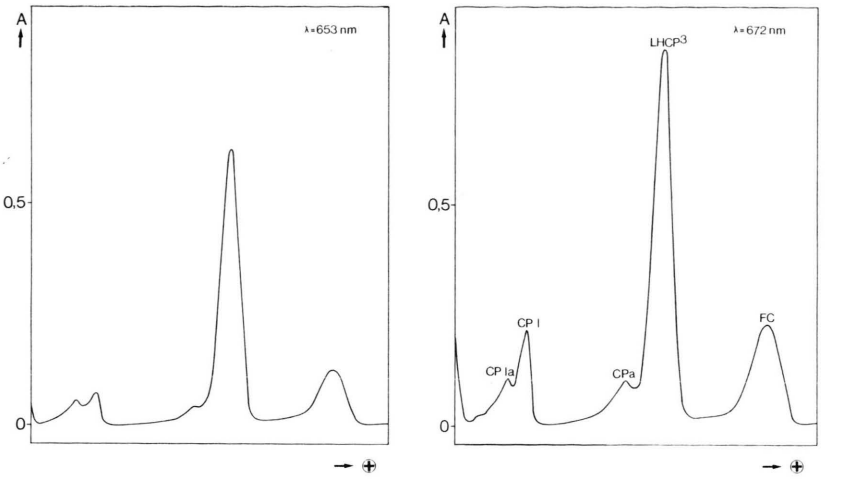

Fig. 5. Gel scans at 672 and 653 nm of Chl containing bands from wild type of *Chlorella fusca*. For solubilization of thylakoids the SDS/Chl ratio was 40.

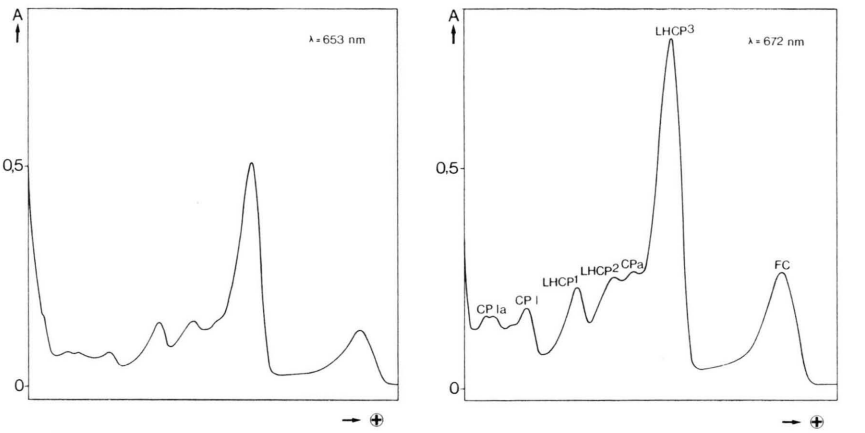

Fig. 6. Gel scans at 672 and 653 nm of Chl containing bands from the normally greened mutant G 10 of *Chlorella fusca*. For solubilization of thylakoids the SDS/Chl ratio was 10.

made an elution and characterization of this complex impossible. The bluish green color and the strong reduction of the densitometric peak at 653 nm already indicate an increased Chl a content. The analysis of scans by means of standard curves shows that in comparison to the neighboring bands the Chl a/Chl b ratio is distinctly increased (Table IV). The CPa-complex of this mutant is extraordinarily labile towards SDS and warmth.

The spectra of LHCP[3] and its oligomers coincide with those of the mutant G 36 (maxima at 671, 653, 470 and 437 nm).

Striking is the relatively high value of the Chl a/Chl b ratio of LHCP[3] and its oligomers.

Concerning the maxima at 674, 437 and 422 nm, the absorption spectrum of the P-700-Chl a-protein complex of the mutant G 10 is identical with that of the normal strain.

Similar to the mutant G 36, weakly greened cells of the mutant G 10 present difficulties in solubilization because of their low chlorophyll content. The SDS/Chl ratio of the normally greened form amounted to 10:1. But in experiments with cultures grown in the dark and exposed to light for 20, 14, 10 or 6 h at the end of the cultivation period, it had to be raised to ratios of 12:1, 15:1, 40:1 and 40:1 respectively. This increase of the SDS concentration leads to an exceeding dissociation of the Chl-proteins. This is expressed in an increase of the free pigment and in a decrease of the especially labile CPa and LHCP-oligomers of this mutant. From algae suspension of a six hour exposure period, only CP I, CP II and a very strong FC-band could be isolated.

Elution and scans provide nearly identical values for the relative proportions of the complexes and the Chl a/Chl b ratio of the normally greened type as

well as of the cells illuminated for 20 or 14 h. There is only a reduction of the CPa-complex. As for the more weakly greened algae, a strong increase of the SDS/Chl ratio contributes to a distinct rise of FC and a complete reduction of CPa and of LHCP-oligomers. In this regard, the values for the Chl a/Chl b ratio of LHCP[3] vary from that of the normally greened type: at an illumination period of 10 h, values ranging from 1,03 to 1,3 were found. In detail, the Chl a/Chl b ratio rose with an increase of the relative CP II-proportion and a decrease of the FC-proportion. Therefore it can be concluded that SDS influenced the Chl a loss of LHCP. Thus a statement about the chlorophyll distribution and the Chl a/Chl b ratio *in vivo* is only possible to a limited extent.

Discussion

By careful solubilization and PAGE, the resolution of chlorophyll-proteins of the normal strain and two mutants of *Chlorella fusca* was made possible. Although the SDS concentration was partly very high, the proportion of FC remained low. Standard curves were used for the characterization of the complexes. By means of these standard curves, the amount of chlorophyll and the Chl a/Chl b ratio can be derived from the scans without eluating the gels. Furthermore, this method allows the determination of the data of Chl-proteins when only small quantities of SDS extract can be used for the electrophoretical separation. A thinning of the eluate would mean an additional complication of the quantitative determination. As the analysis of the densitometric tracing is based on the peak maximum, mistakes in elution – caused by the overlapping of neighboring bands – can easily be avoided. This is especially important to the measurement of the Chl a/Chl b ratio of CPa-complex and of CP I. Bands migrating closely together can often be characterized by means of standard curves although a separation by elution is impossible.

Besides the P-700-Chl a-protein, the light-harvesting Chl a/b-protein, and the free pigment, the normal strain and the mutants of *Chlorella fusca* show additional bands after PAGE resolution.

There is a least one band – migrating between the top of the gel and CP I – which shows an absorption spectrum similar to that of CP I but whose Chl b content is essentially higher. Increase of SDS/Chl ratio results in a reduction of the band in question. Some authors describe Chl-protein complexes migrating between the starting line and CP I, but all these complexes possess a very high Chl a/Chl b ratio [9–11]. The high Chl b content of CP Ia of *Chlorella fusca* indicates the presence of LHCP. Thus this complex could be an aggregate of P-700 Chl a-protein and LHCP corresponding to the aggregates of PS I and LHCP postulated by Staehelin *et al.* [36] in their membrane model. Formerly the presence of small amounts of the light-harvesting Chl a/b-protein was repeatedly demonstrated in PS I particles prepared with digitonin or by physical methods [7]. The Chl b-containing component could possibly represent a specific PS I-light-harvesting complex (ACP I or LHCP I), which Boardman *et al.* [3] postulate in one of their models for the organization of the Chl-protein complexes in the photosynthetic unit of higher plants.

Because of a very similar electrophoretic mobility of the CP Ia and the CP I of the mutant G 10 and the normal strain of *Chlorella fusca*, their separation is very difficult. But by means of a standard curve it could be shown that CP I complex is extremely rich in Chl a. If a pure complex is obtained – as shown with the mutant G 36 – only Chl a can be proved.

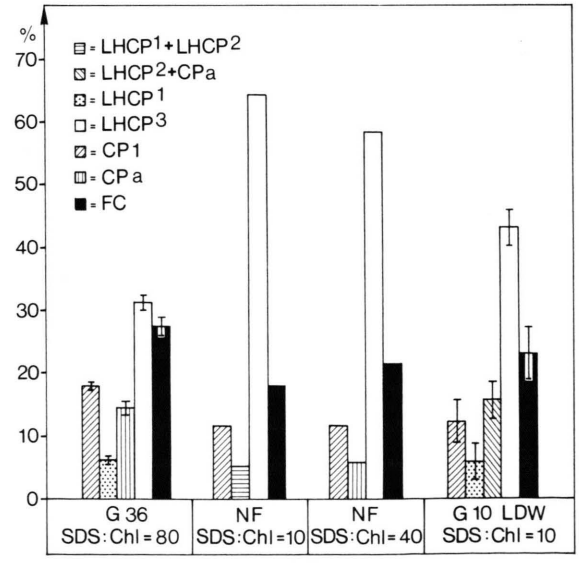

Fig. 7. Relative proportions of Chl-protein in the normal strain and two mutants of *Chlorella fusca* measured by densitometer tracings of polyacrylamide gels of SDS extracts. At a SDS/Chl ratio of 10, the CPa could not be separated from LHCP[3] of the normal form.

It is striking that the amount of CP I of the mutant G 36 is markedly higher than that of the other strains, although it contains only Chl a (*cf.* Fig. 7). In contrast to G 36, the percentage of CP I of the normal strain and of the mutant G 10 (being normally greened or illuminated for 20, 14, or 10 h, respectively) is quite similar.

The same applies to the absorption spectra of P-700-Chl a-proteins: The normal strain and the mutant G 10 show identical absorption spectra, whereas the mutant G 36 is characterized by a remarkable shifting of the maximum in the red to longer wavelengths. The primary developing stage of the thylakoids of this yellow mutant could be responsible for such a shifting [28]. In this context Hiller *et al.* [37] postulate a first and a second stage of CP I synthesis. An absorption maximum at 676 nm of the CP I complex – being isolated from barley chloroplasts of flashed leaves by hydroxylapatite chromatography – together with strongly reduced Chl/P-700 ratio can be considered as a feature of a developing stage corresponding to "the earliest stages of greening when the leaves are exposed to alternate light/dark periods".

In the electrophoretograms, 1–2 bands appear, migrating between CP I and LHCP^3; congruity of the absorption spectra provides evidence of their being oligomers of CP II. Whereas the normal type and the mutant G 10 show two of such bands, G 36 (SDS/Chl = 80) has only one band, whose R_f-value is in keeping with that of LHCP^1 of the normal strain and of G 10. Striking is the small proportion of LHCP di- and oligomers in the normal type at low SDS concentrations (SDS/Chl = 5), whereas the mutant G 10 shows a high proportion (*cf.* Fig. 7). The oligomers of LHCP^3 are more sensitive to warmth and high SDS concentrations than LHCP^3 itself. This observation is in accordance with results reported in literature [10, 16]. With a low SDS/Chl quotient, the mutant G 36 shows an additional band between CPa and LHCP^1, a band which does not seem to be an oligomer of LHCP^3 on account of its high Chl a/Chl b ratio of 5 (at SDS/Chl = 40).

The light-harvesting Chl a/b-protein complexes of the three strains of *Chlorella fusca* show very similar spectral properties. But there are great differences in the Chl a/Chl b ratio: The value for the normal strain is 1.3; for the mutants it is essentially higher (1.6 to 1.85). This observation is contradicted in literature, where generally equimolar quantities in Chl a and Chl b of CP II are reported [4, 7, 13]. According to Kan and Thornber [14], the LHCP of the green alga *Chlamydomonas reinhardii* is supposed to have a Chl a/Chl b ratio of 1:1. But frequently higher values for the Chl a/Chl b quotient were found, too [1, 2, 38, 39]. Thus, quick electrophoresis in low temperature or low SDS/Chl ratios during extraction result in Chl a/Chl b ratios of 1.3 to 1.8 [3].

The Chl a/Chl b ratio of LHCP^3 of the normal strain of *Chlorella fusca* (1.3) is in perfect agreement with the value (1.28) described by Anderson *et al.* [10] and Boardman and Anderson [9] for spinach as well with the value reported by Wild and Krebs [34] for *Sinapis alba* (1.26). Remy *et al.* [18] suppose that the Chl a/Chl b ratio of the *in vivo* CP II is 1.25. Arntzen and Ditto [40] found a Chl a/Chl b ratio of 1.3 in a LH-fraction, which had been resolved from the membrane of *Pisum sativum* by digitonin and had been separated by density gradient centrifugation. The essentially higher value of the mutants may be explained by their relative Chl b deficiency.

In contrast to studies of Boardman and Anderson [9] but in accordance with results of Remy *et al.* [18], we found that the Chl a/Chl b ratios of the di- and oligomers of LHCP were higher than that of LHCP^3. In a reelectrophoresis of the oligomers, Remy *et al.* observed a loss of Chl a, which could be found in the FC-zone [18]. These results lead to the conclusion that the Chl a/Chl b ratio of the *in vivo* LHCP complexes is higher than 1 and can certainly assume different values.

In regard to the relative proportion of LH-Chl a/b-protein complexes, the three strains of *Chlorella fusca* vary in a characteristic way. This difference is illustrated in Fig. 7, in which the relative proportions of Chl-protein complexes are compared. A relationship between the LH percentage and the Chl b content of the plant can be established. The Chl a/Chl b ratio of the normal type is essentially lower than that of the mutants. The lower Chl a/Chl b ratio of the normal type results in a high proportion of LHCP which corresponds to a high Chl b content. In contrast to this, the Chl a/Chl b ratio of the yellow mutant G 36 is strongly increased, corresponding to a considerable reduction of LHCP. A relationship between the Chl b and the relative proportion of LHCP of plants is described for a wide variety of plants [7, 9, 10, 12, 17, 20, 41, 42].

Between LHCP[3] and its oligomers migrates the CPa complex. It is very sensitive to warmth in the three *Chlorella*-strains examined. Whereas the complex of the mutant G 10 is extremely labile in regard to SDS, high detergent concentrations were needed to produce a distinct band in the electrophoresis in the normal type. In spite of the high SDS concentration (necessary for solubilization) the complex of the mutant G 36 is surprisingly stable. Despite this stability, the SDS incubation period and electrophoresis were kept short.

Only in the mutant G 36 – with its strongly reduced LHCP complex due to its Chl b deficiency – the separation of the CPa complex is possible in such a way that it can be obtained in a very pure state. Thus it can be used for characterization. The maxima of the absorption spectrum at 671 and 437 nm are in correspondence with those of LHCP, but all the typical Chl b shoulders are missing. Thus the identification of the complex with CP I, CP II or the free pigment is excluded. From the similarity of the absorption spectra and the same position in the electrophoretograms one can conclude that the CPa-complex of *Chlorella fusca* is identical with "band IV" of maize [20], with "band A" of *Lactuca* [22], with "band II b_2" of *Nicotiana* [11] and with the "CPa-band" [10] or "39–42 Kdalton-band" [24] respectively of spinach. It is true that, according to the absorption spectra, all these authors suppose that this complex only contains Chl a. But so far it could not be plainly proved. Anderson *et al.* [10] found different values for the Chl a/Chl b ratio; Boardman and Anderson [9] determined a value of 3.66 for spinach. Other authors did not publish any data.

In regard to the normal strain and the mutant G 10, the overlapping of the complex with the neighboring bands results in relatively low Chl a/Chl b ratios. The CPa-complex of the normal type – where a better separation of the LHCP[3]-band is attained by increasing SDS/Chl ratios – clearly shows that the insuffient separation (short electrophoretic distances) is responsible for the high Chl b contents. In comparison with the normal type, the complex of the mutant G 36, which can be obtained in markedly absolute purity, contains Chl a only. This could plainly be proved by the analysis of the densitometric tracings.

For the normal form, the relative proportion of the CPa-complex is 6% (SDS/Chl = 40). This value corresponds to those described for *Lactuca sativa* (5% [22]) and for barley (8% [9]). For a barley mutant which lacked both the Chl a and the LH complex, the CPa possesses 25% of the Chl [9]. The finding that the relative proportion of CPa of the mutant G 36 amounts to 15% must be considered a high percentage because the CP II accounts for 32%. Hayden and Hopkins [20] could only determine a proportion of 8–10% for this complex of a Chl b-deficient maize mutant. According to the fact that mutants with a strongly diminished CP II content do not show a reduction of the complex IV (CPa), the authors exclude a connection of the complex with LHCP. As this applies also to the Chl b-deficient mutant of *Chlorella fusca*, the suggestion of the CPa-complex being a good candidate for the PS II reaction center is supported. It is strengthened by the fact that this complex is completely free of Chl b. This was proved for the mutant G 36 of *Chlorella fusca*. Numerous separation of subchloroplast fractions either show a high Chl a/Chl b ratio for the PS II or only show Chl a [43–46].

Acknowledgement

This work was supported by the Deutsche Forschungsgemeinschaft.

[1] T. Ogawa, F. Obata, and K. Shibata, Biochim. Biophys. Acta **112,** 223–234 (1966).
[2] J. P. Thornber, R. P. F. Gregory, C. A. Smith, and J. Legett Bailey, Biochemistry **6,** 391–396 (1967).
[3] N. K. Boardman, J. M. Anderson, and D. J. Goodchild, Current Topics in Bioenergetics, **Vol. 8** (D. R. Sanadi and L. P. Vernon, eds.), pp. 36–109, Academic Press, New York-San Francisco-London 1978.
[4] J. P. Thornber and R. S. Alberte, Photosynthesis I, Encyclopedia of Plant Physiology, New Series, **Vol. 5,** (A. Trebst and M. Avron, eds.), pp. 574–582, Springer, Berlin-Heidelberg-New York 1977.
[5] R. S. Alberte and J. P. Thornber, FEBS Lett. **91,** 126–130 (1978).
[6] N. E. Dietrich and J. P. Thornber, Biochim. Biophys. Acta **245,** 482–493 (1971).
[7] J. P. Thornber, Ann. Rev. Plant Physiol. **26,** 127–158 (1975).
[8] J. S. Brown, R. S. Alberte, and J. P. Thornber, Proc. 3rd Intern. Congr. Photosynthesis, **Vol. III,** (M. Avron, ed.), pp. 1951–1962, Elsevier, Amsterdam-Oxford-New York 1975.
[9] N. K. Boardman and J. M. Anderson, Chloroplast Development, (G. Akoyunoglou and J. H. Argyroudi-Akoyunoglou, eds.), pp. 1–14, Elsevier, Amsterdam-Oxford-New York 1978.
[10] J. M. Anderson, J. C. Waldron and S. W. Thorne, FEBS Lett. **92,** 227–233 (1978).
[11] R. Remy and J. Hoarau, Chloroplast Development, (G. Akoyunoglou and J. H. Argyroudi-Akoyunoglou, eds.), pp. 235–240, Elsevier, Amsterdam-Oxford-New York 1978.
[12] J. P. Thornber and H. R. Highkin, Eur. J. Biochem. **41,** 109–116 (1974).
[13] J. P. Thornber, R. S. Alberte, F. A. Hunter, J. A. Shiozawa, and K. S. Kan, Brookhaven Symposia in Biology **28,** 132–148 (1977).
[14] K. S. Kan and J. P. Thornber, Plant Physiol. **57,** 47–52 (1976).
[15] P. A. Armond and C. J. Arntzen, Plant Physiol. **59,** 398–404 (1977).
[16] F. Hermann and A. Meister, Photosynthetica **6,** 177–182 (1972).
[17] S. Genge, D. Pilger, and R. G. Hiller, Biochim. Biophys. Acta **347,** 22–30 (1974).
[18] R. Remy, J. Hoarau, and J. C. Leclerc, Photochem. Photobiol. **26,** 151–158 (1977).
[19] D. B. Hayden and W. G. Hopkins, Can. J. Bot. **54,** 1684–1689 (1976).
[20] D. B. Hayden and W. G. Hopkins, Can. J. Bot. **55,** 2525–2529 (1977).
[21] E.-M. Aro and N. Valanne, Physiol. Plant. **43,** 261–265 (1978).
[22] F. Henriques and R. B. Park, Biochem. Biophys. Res. Commun. **81,** 1113–1118 (1978).
[23] K. R. Miller, G. J. Miller, and K. R. McIntyre, J. Cell Biol. **71,** 624–638 (1976).
[24] J. S. C. Wessels and M. T. Borchert, Biochim. Biophys. Acta **503,** 78–93 (1978).
[25] S. Bendix and M. B. Allen, Arch. Mikrobiol. **41,** 115–141 (1962).
[26] A. Wild, Progress in Photosynthesis Research, **Vol. II,** (H. Metzner, ed.), pp. 871–876, Metzner, Tübingen.
[27] A. Wild, H.-O. Zickler, and H. Grahl, Planta **97,** 208–223 (1971).
[28] K. Bauer and A. Wild, Z. Pflanzenphysiol. **80,** 443–454 (1976).
[29] A. Wild and K.-H. Fuldner, Planta **136,** 281–282 (1977).
[30] A. Wild, U. Trostmann, J. Kietzmann, and K.-H. Fuldner, Planta **140,** 45–62 (1978).
[31] A. Wild and K. Egle, Biol. Zentralblatt **86** (Suppl.) 495–508 (1967).
[32] A. Wild, K. Bauer, and O. Sonnenberger, Z. Pflanzenphysiol. **72,** 148–162 (1974).
[33] R. Ziegler and K. Egle, Beitr. Biol. Pflanzen **41,** 11–37 (1965).
[34] A. Wild, B. Krebs, and W. Rühle, Z. Pflanzenphysiol., in press.
[35] J. M. Anderson, Biochim. Biophys. Acta **416,** 191–235 (1975).
[36] L. A. Staehelin, P. A. Armond, and K. R. Miller, Brookhaven Symposia in Biology **28,** 278–315 (1976).
[37] R. G. Hiller, T. B. G. Pilger, and S. Genge, Chloroplast Development, (G. Akoyunoglou and H. J. Argyroudi-Akoyunoglou, eds.), pp. 215–220, Elsevier, Amsterdam-Oxford-New York 1978.
[38] S. D. Kung and J. P. Thornber, Biochim. Biophys. Acta **253,** 285–289 (1971).
[39] R. S. Alberte, J. P. Thornber, and W. Naylor, J. Exp. Bot. **23,** 1060–1069 (1972).
[40] C. J. Arntzen and C. L. Ditto, Biochim. Biophys. Acta **449,** 259–274 (1976).
[41] J. S. Brown, R. S. Alberte, J. P. Thornber, and C. S. French, Carnegie Institution Washington Year Book **73,** 694–706 (1974).
[42] O. Machold, A. Meister, H. Sagromsky, G. Hoeyer-Hansen, and D. von Wettstein, Photosynthetica **11,** 200–206 (1977).
[43] L. P. Vernon, E. R. Shaw, T. Ogawa, and D. Raveed, Photochem. Photobiol. **14,** 343–357 (1971).
[44] J. S. C. Wessels, O. van Alphen-van Waveren, and G. Voorn, Biochim. Biophys. Acta **292,** 741–752 (1973).
[45] S. M. Klein and L. P. Vernon, Plant Physiol. **53,** 777–778 (1974).
[46] K. Satoh and W. Butler, Plant Physiol. **61,** 373–379 (1978).