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Changes in the abundance of individual apoproteins of light-harvesting chlorophyll *a*/*b*-protein complexes of Photosystem I and II with growth irradiance in the marine chlorophyte *Dunaliella tertiolecta*

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The marine chlorophyte *Dunaliella tertiolecta* responds to increased growth irradiance (in the range 70–1 900 μmol quanta · m^{-2} · s^{-1}) by decreasing its cellular content of reaction centers I and II by as much as 75% (Sukenik, A., Bennett, J. and Falkowski, P.G. (1987) Biochim. Biophys. Acta 891, 205–215). We have used Western blot techniques and antibodies raised against homologous pea proteins to examine changes in the concentration of the corresponding light-harvesting antenna complexes, LHC I and LHC II. Three LHC I apoproteins (22, 27.5 and 32 kDa) and four LHC II apoproteins (24.5, 28.5, 30 and 31 kDa) were detected. With increasing growth irradiance, the 22 and 27.5 kDa LHC I apoproteins and the 30 and 31 kDa LHC II apoproteins declined in parallel with RC I and RC II. In contrast, the 32 kDa LHC I apoprotein remained equally abundant per cell at all irradiances and the 28.5 kDa II apoprotein declined by only 30%. The fact that purified LHC II extracted from high-light cells (1 200 μmol quanta · m^{-2} · s^{-1}) contained a lower chlorophyll (Chl) *b* content than LHC II purified from low-light cells (70 μmol quanta · m^{-2} · s^{-1}) suggests that the LHC II apoproteins differ in their capacity to bind Chl *b*. We conclude that photo-adaptive changes in pigment composition in *D. tertiolecta* are brought about not only by changes in the total abundance of LHC I and LHC II, but also by differential accumulation of individual LHC I and LHC II apoproteins. A simple procedure for detecting irradiance-dependent changes in the light-harvesting apparatus of other green algae is presented.

Introduction

The photosynthetic apparatus of oxygenic photoautotrophs consists of two photosystems, each of which contains three major pigment-binding protein complexes: a reaction center, core antenna complexes, and peripheral antenna complexes [1]. Most photosynthetic organisms adjust the relative abundance of their pigment-protein complexes in response to growth irradiance levels [2–5]. This phenomenon, variously called photoadaptation, light-shade adaptation or sun-shade adaptation, is thought to be due to the differential synthesis and/or degradation of specific pigment-protein complexes triggered by changes in photon flux densities. Changes in the relative abundance of pigment-protein complexes may alter the effective absorption cross section of one or both photosys-

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Abbreviations: LHC I and LHC II; light-harvesting chlorophyll a/b-protein complex of Photosystem I and of Photosystem II, respectively; Chl, chlorophyll; RC, reaction center; HL, high light; LL, low light; PS II, Photosystem II; PAGE, polyacrylamide gel electrophoresis.

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tems leading to an increased ability to harvest light at low photon flux densities and a decreased chance of causing photodamage to the photosynthetic apparatus at high photon flux densities [6,7]. The exact mechanisms responsible for these changes are unknown, but they do not appear to be mediated by photochrome [4,8].

In higher plants and green algae, the pigment-protein complexes which function as the peripheral antenna for the reaction centers invariably contain Chl *a* and *b* [1,7]. The Chl *a/b* ratio within the pigment protein complexes seems to be highly conserved. The most abundant pigment protein complex is LHC II, a light-harvesting complex with a Chl *a/b* ratio usually reported to be between 1.0 and 1.2 [1], which accounts for 40–60% of total Chl of green plants. A second pigment protein complex, LHC I, is reported to have a Chl *a/b* ratio of 3.6 and accounts for 30–40% of the total Chl of green plants [1]. Both LHC II and LHC I consist of a number of closely related apoproteins, which are encoded by nuclear gene families [9,10].

Dunaliella tertiolecta is a green alga that is well known for its ability to adapt to changes in growth irradiance levels primarily by increasing or decreasing the number of reaction centers, while the ratio of reaction centers and total number of Chl molecules serving each reaction center remain relatively constant [11–13]. However, changes in total cellular Chl levels are accompanied by large changes in Chl *a/b* ratios, which are usually interpreted as changes in the number of antenna pigment proteins serving the reaction centers, especially LHC II [4]. On the surface, these two results are contradictory. However, we reported elsewhere [14] that LHC II complexes isolated from cells grown under high light (HL, 1 200 μmol quanta $\cdot m^{-2} \cdot s^{-1}$) and low light (LL, 70 μmol quanta $\cdot m^{-2} \cdot s^{-1}$) differed with respect to Chl *b* content. HL complexes contained on average 5 Chl *a*, 1 Chl *b* and two lutein molecules per apoprotein, while LL complexes contained on average 5 Chl *a*, 2 Chl *b*, and two lutein molecules per apoprotein. There are two ways of explaining these differences: (a) HL and LL complexes contain the same apoproteins in the same relative concentrations, with each apoprotein being able to change its Chl *b* content depending on growth irradiance, or (b) each LHC II apoprotein has a fixed characteristic pigment-binding capacity but by changing the proportions of its apoproteins a cell may alter its average pigment composition in response to growth irradiance. We experimentally examined this second possibility by using Western blotting and polyclonal antibodies raised against pea LHC II to determine the cellular contents of four LHC II apoproteins as a function of growth irradiance. To provide a more complete analysis of the response of the light-harvesting proteins of *Dunaliella,* we used the same approach to analyze the cellular contents of three LHC I apoproteins. The results of this study suggest that variations in cellular Chl *a/b* ratios do not simply reflect changes in the abundance of light-harvesting antenna proteins relative to reaction centers, but also reflect changes in the abundance of specific LHC apoproteins, which may have differing pigment compositions.

Materials and Methods

Culture conditions. D. tertiolecta (Woods Hole clone DUN) was maintained at 18 °C under steady-state growth conditions in 3.2 l turbidostats with natural sea water enriched with *f/2* nutrients as previously described [11,15]. Light was provided continuously by banks of very high output fluorescent tubes and cells were grown at five irradiance levels: 70, 200, 700, 1 200 and 1 900 μmol quanta $\cdot m^{-2} \cdot s^{-1}$. The cultures were kept optically thin ($(3–5) \cdot 10^5$ cells/ml) to minimize self shading. Growth irradiance levels were measured in the turbidostats with a Biospherical Instruments QSL100 scalar quantum sensor (400–700 nm). At all growth irradiance levels nutrient concentrations were saturating. The pH of the sea water was buffered at 7.8 and the cultures were continuously bubbled with air; growth was never limited by the availability of CO_2 at these low cell densities. During steady-state growth at each light level the cultures were sampled for pigment content, oxygen flash yields, and photosynthetic characteristics. In addition, large volume samples (about 2.0 l) were concentrated by continuous centrifugation, flash frozen in liquid N_2 and stored at −60 °C for analysis of chloroplast proteins.

Pigment analyses. Photosynthetic pigments were extracted by homogenizing samples filtered on glass fiber filters in 90% acetone. Corrected spectra were recorded on an Aminco DW-2a spectrophotometer and Chl's *a* and *b* were calculated using the equations of Jeffrey and Humphrey [16]. Total carotenoid concentrations were calculated using an extinction coefficient of 2500 $mM^{-1} \cdot cm^{-1}$ at 480 nm. Additionally, pigments were extracted in 80% methanol and separated by isocratic elution by high performance liquid chromatography on a 3 μm bead, 7.5 cm RP-8 column with 95:5 (v/v) methanol/water. Pigments were detected by absorbance at 438 nm and by fluorescence using a Corning 2-64 excitation filter and a 680 nm interference filter. Pigments were quantified by areal integration with an Hewlett-Packard HP 3392A digital integrator and verified with pure standards isolated from spinach and *D. tertiolecta* by thin layer chromatography.

Measurement of reaction centers. Oxygen flash yields were measured with a Rank Brothers electrode using synchronized GenRad Stroboslave 1539A xenon flash bulbs as previously described [12]. The cellular concentration of RC II was calculated from cellular Chl concentrations and the flash yields assuming that each oxygen produced required four light-driven, one-electron oxidation steps, each with a quantum yield of unity [17,18].

P-700 was measured by light induced absorption difference spectra in membranes solubilized in 0.01% Triton X-100 containing 10 mM $MgCl_2$ as previously described [11,12].

Identification of LHC I and LHC II. The cellular content of LHC I and LHC II was determined by autoradiographs of Western blots of total soluble cell protein after separation by SDS-polyacrylamide gel electrophoresis. About $5 \cdot 10^8$ cells were concentrated by centrifugation and resuspended in 1 ml of an homogenizing buffer (100 mM Na_2CO_3, 100 mM dithiothreitol and 200 μM PMSF). The suspension was sonicated for 90 s at 4° C with a Kontes microprobe sonicator. SDS and glycerol were added to give final concentrations of 0.5% and 10%, respectively and a SDS/Chl (w/w) ratio of 15:1. The sample was gently stirred at room temperature for 10 min and centrifuged at 20000 $\times g$ for 10 min. Proteins in the supernatant were denatured by boiling for 2 min, 35 μl aliquots were separated by electrophoresis on a 10% polyacrylamide gel slab using the buffers of Laemmli [19], and the proteins were transferred to nitrocellulose by electrophoresis as described [20]. LHC I and LHC II polypeptides were detected by successive incubation of the nitrocellulose with polyclonal antibodies raised against pea LHC I [21] or LHC II [22] and ^{125}I-labeled protein A. The antibodies raised against LHC I did not cross-react with LHC II apoproteins and vice versa [21]. Autoradiograms were scanned to provide quantitation. LHC II isolated from *D. tertiolecta* was used to establish a quantitative calibration. A linear relationship was established between the volume of cell extract loaded onto gels and the product of (autoradiograph peak area) $\times$ (band width) [22].

Thylakoid preparation and LHC II isolation. Thylakoids were prepared as previously described [23]. All buffers contained 200 μM PMSF. Cells and chloroplasts were ruptured in 0.4 M sucrose and centrifuged at 30000 $\times g$ for 20 min. The pellet was resuspended and washed three times in 6.2 mM Tris, 48 mM glycine (pH 7.8), containing 2 mM EDTA, to give 0.5 mg Chl/ml. Membranes were partially solubilized in 60 mM octyl-β-D-glucopyranoside for 45 min at room temperature as described by Camm and Green [24]. Undissolved membranes were removed by centrifugation at 200000 $\times g$ for 1 h. The solubilized material was loaded on a 0.1–0.5 M linear sucrose gradient containing 2 mM Tris-maleate buffer (pH 7.0)/2 mM EDTA/30 mM octyl-β-D-glucopyranoside. The gradients were centrifuged for 16 h at 200000 $\times g$ and the highly fluorescent, upper green band, which is enriched with LHC II, was collected. LHC II was further purified by precipitation with 10 mM $MgCl_2$ and centrifuged at 30000 $\times g$ on a 1 M sucrose cushion as described by Burke et al. [25]. The pellet was resuspended in 2 mM Tris-maleate (pH 7.0) with 2 mM EDTA and 30 mM octyl-β-D-glucopyranoside and analyzed for apoprotein composition, pigment content and amino acid composition. Amino acids were analyzed according to the general procedures of Spackman et al. [26], following hydrolysis in vacuo in 6 M HCl at 110° C.

Octyl-β-D-glucopyranoside-solubilized mem-

branes were loaded onto 1.5 mm thick LiDS polyacrylamide gels containing 3 mM octyl-β-D-glucopyranoside [24]. The gel was run at 4°C for 2.8–4 h at a constant current of 10 mA. The pigmented bands resolved on that gel were immediately photographed, cut from the gel, placed between glass plates and scanned with an SLM Aminco DW2c spectrophotometer. Pigment proteins in each of the gel slices were eluted by diffusion overnight in 500 μl of 0.1% LiDS at 4°C in the dark. The eluted pigments were extracted by sonication into 100 μl isobutanol, and separated by centrifugation. The isobutanol phase was removed and the pigment composition was analyzed by HPLC as described above.

Phosphorylation of LHC II. To investigate phosphorylation of thylakoid membrane proteins in vitro membranes were prepared from LL cells as previously described [25]. The thylakoids were resuspended in 6.2 mM Tris/48 mM glycine/10 mM $MgCl_2$/10 mM NaF to give a final concentration of 0.5 mg total Chl/ml. 500 μl aliquots of the suspended thylakoids were equilibrated at 20°C in a final volume of 1 ml of the same buffer. Equilibration took place in the light (1000 μmol quanta $\cdot m^{-2} \cdot s^{-1}$). Phosphorylation reactions were started by addition of [γ-^{32}P]ATP (10 Ci per mol) at a final concentration of 100 μM. The reaction was stopped by addition of 0.5 ml of cold trichloroacetic acid (30% w/v). Thylakoid membranes were washed several times with the same buffer containing trichloroacetic acid (5% w/v) and finally resuspended in the Tris-glycine buffer and dissolved in 10% SDS. Proteins were separated on 10% SDS-PAGE and ^{32}P-labeled proteins were visualized after autoradiography.

Results

Pigment-protein complexes of Dunaliella

The photosynthetic pigments of *Dunaliella tertiolecta* include Chl *a*, Chl *b*, lutein and several other carotenoids. As cells adapted to irradiance levels between 80 and 1900 μmol quanta $\cdot m^{-2} \cdot s^{-1}$, the cellular Chl *a* content varied 6-fold (Table I). The cellular concentrations of the major accessory pigments, Chl *b* and lutein, followed the same trend as Chl *a* but Chl *b* varied 15-fold, while lutein varied only 2.4-fold over the same range of irradiance. Consequently, both the Chl *a*/Chl *b* ratio and the lutein/Chl *b* ratio increased with increasing growth irradiance (Table I). Since Chl *b* is located mainly in LHC II, these results for high light intensities could be explained either by the disappearance of LHC II or by the accumulation of a novel form of LHC II that binds little or no Chl *b*. Our isolation of a Chl *b*-deficient LHC II complex from HL cells of *D. tertiolecta* [14] provides clear support for the latter explanation. However, our initial attempts to visualize such a complex on SDS or LiDS-polyacrylamide gel electrophoresis were unsuccessful. It proved to be more labile than LHC II from LL cells. Preservation of the complex was achieved when thylakoids were solubilized with octyl-β-D-glucopyranoside rather than SDS or LiDS prior to electrophoresis.

We analyzed changes in the pigment-protein complexes in the thylakoid membranes from *D. tertiolecta* growth at 70 μmol quanta $\cdot m^{-2} \cdot s^{-1}$ (LL) and 1200 quanta $\cdot m^{-2} \cdot s^{-1}$ (HL). Thylakoids were solubilized in 60 mM octyl-β-D-glucopyranoside and separated by LiDS-polyacrylamide gel electrophoresis at 4°C (Fig. 1). Under these mildly denaturing conditions several green complexes were resolved and identified according to their mobility and absorption spectra following the nomenclature of Anderson [27]. Three CP1 bands and a single CPa band, corresponding to complexes derived from RC I and RC II, respectively, were found in both LL and HL membranes. Two Chl *a*/*b* protein complexes corresponding to monomeric and oligomeric forms of LHC II (LHCP3 and LHCP1) were resolved from LL thylakoids, but only the LHCP3 band was resolved from HL thylakoids; LHCP1 could not be detected by absorption or even by fluorescence on the green gels from HL thylakoids. For both HL and LL samples, the LHCP complexes contained 45–54% of total Chl *a*. The free-pigment fraction contained 14–20% of total Chl *a*, mostly (> 90%) in the form of chlorophyllide *a*, as measured by HPLC. The free pigment zone also contained substantial amounts of lutein but very little Chl *b*.

The ratio of Chl *a* recovered in LHC II (LHCP1 and LHCP3) to that recovered in CPa is approximately equal for both LL and HL cells (Fig.

TABLE I

THE EFFECT OF GROWTH IRRADIANCE LEVEL ON CELLULAR PIGMENTATION IN *DUNALIELLA TERTIOLECTA*

Growth irradiance (μmol quanta·m^{-2}·s^{-1})	Chl *a*	Chl *b*	lutein	Chl *a* / Chl *b*	lutein / Chl *a*	lutein / Chl *b*
	(10^{-16} mol per cell)			(mol/mol)		
70	22.8	4.75	7.9	4.8	0.35	1.66
200	19.3	2.24	5.5	6.2	0.40	2.46
700	6.9	0.87	4.3	7.9	0.62	4.94
1200	5.2	0.61	3.8	8.5	0.73	6.23
1900	3.5	0.31	3.3	11.2	0.94	10.7

1). Taking the data for HL thylakoids from Fig. 1, and assuming that CPa represents an RC II core and contains 50 Chl *a* as found for spinach [28], we calculate that there are about 225 LHC II Chl *a*'s per RC II. We have shown elsewhere [14] that LHC II from *Dunaliella* contains on average about 5 Chl *a*'s per apo-LHC II, so there must be be about 45 LHC II apoproteins per RC II at HL. The calculation for LL thylakoids yields about 250 LHC II Chl *a* per RC II, or about 50 LHC II apoproteins per RC II at LL.

Apoproteins of light-harvesting complexes in Dunaliella

Since LHC I is far less stable than LHC II during electrophoresis [29], we compared the behaviour of LHC I and LHC II at the apo-protein level. Polyclonal antibodies raised against pea LHC I apoproteins were used to study changes in total levels of LHC I and in the levels of individual apoproteins (Fig. 2). Three LHC I apoproteins were detected, with molecular masses of 22, 27.5 and 32 kDa, of which the last was most abundant (Table II). Fig. 3 compares the irradiance responses of the levels of the LHC I apoproteins with that of RC I and LHC II. The data reveal that, despite marked changes in cellular Chl, the cellular abundance of LHC I was relatively con-

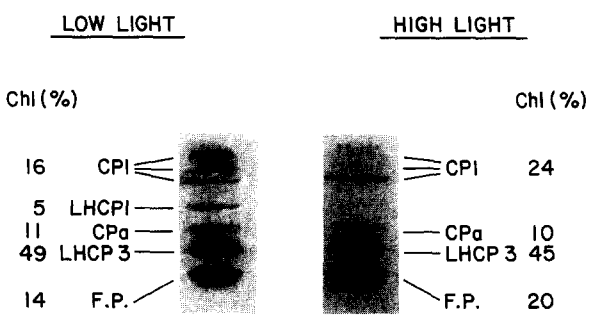

Fig. 1. Pigment-protein complexes from thylakoid membranes of *D. tertiolecta*. Membranes from cells grown at 70 (LL) and 1200 (HL) μmol quanta·m^{-2}·s^{-1} were dissolved in 60 mM octyl-β-D-glucopyranoside and analyzed by mildly denaturing LiDS-PAGE (10% acrylamide) at 40 °C and photographed without staining. 33 μg Chl $a+b$ from LL membranes with a Chl a/b ratio of 5.2, and 16 μg Chl $a+b$ from HL membranes with a Chl a/b ratio of 9.5 were loaded in the respective lanes. Numbers shows Chl *a* content of each band as percentage of total Chl *a* of sample. F.P., free pigment.

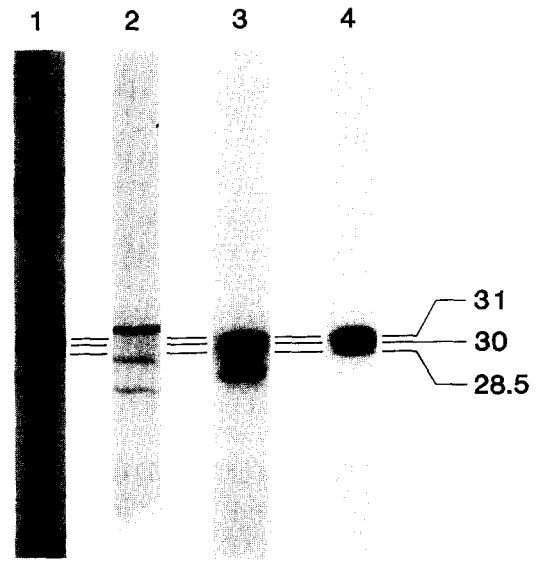

Fig. 2. Characterization of LHC I and LHC II apoproteins from LL *D. tertiolecta*. Lane 1: Coomassie blue-stained proteins extracted from thylakoid membranes and resolved by SDS-PAGE (15% acrylamide). Lane 2: Western blot of LHC I apoproteins. Lane 3: Western blot of LHC II apoproteins. Lane 4: Autoradiograph of ^{32}P-labeled thylakoids from LL cells.

TABLE II

RELATIVE ABUNDANCE OF LHC I AND LHC II APOPROTEINS OF *DUNALIELLA TERTIOLECTA* GROWN AT 70 AND 1900 μMOL QUANTA·m^{-2}·s^{-1}

Apoproteins were detected by Western blotting of thylakoid proteins. Abundance of each apoprotein is expressed as a percentage of total LHC I or LHC II protein at each irradiance.

Molecular mass (kDa)	Percent apoprotein abundance	
	$I = 70$	$I = 1900$
LHC I		
22	25	10
27.5	26	14
32	49	76
LHC II		
24.5	20	19
28.5	15	49
30	25	19
31	40	13

stant. As RC I/cell varies in parallel with cellular Chl, LHC I/RC I varied significantly, and was paralleled by changes in ratio of LHC I/LHC II. Between 70 and 1900 μmol quanta · m^{-2} · s^{-1}, the 22 and 27.5 kDa apoproteins delcined by about 70%, approximately in parallel with RC I, while the 32 kDa apoprotein remained constant in cellular concentration. We did not purify LHC I from *D. tertiolecta*, and consequently cannot calculate the absolute number of LHC I apoproteins per RC I or the number of LHC I molecules per RC I.

The number of LHC II apoproteins per RC II was determined from Western blots using polyclonal antibodies raised against pea LHC II. Autoradiographs of LHC II apoproteins reveal four different polypeptides with apparent molecular masses of 24.5, 28.5, 30 and 31 kDa (Fig. 2). The relative abundance of these proteins is indicated in Table II. As growth irradiance increased, the relative abundance of LHC II apoprotein bands decreased on a cell number basis (Fig. 4). Taken together, the total LHC II apoprotein content per cell decreased by about 75% between 70 and 1900 μmol · m^{-2} · s^{-1}, but since the RC II content per cell declined by a similar percentage, the LHC/RC II ratio remained approximately constant, at 55–60 LHC II apoproteins for RC II (Fig. 4). The results in Figs. 1 and 4 both indicate that the total

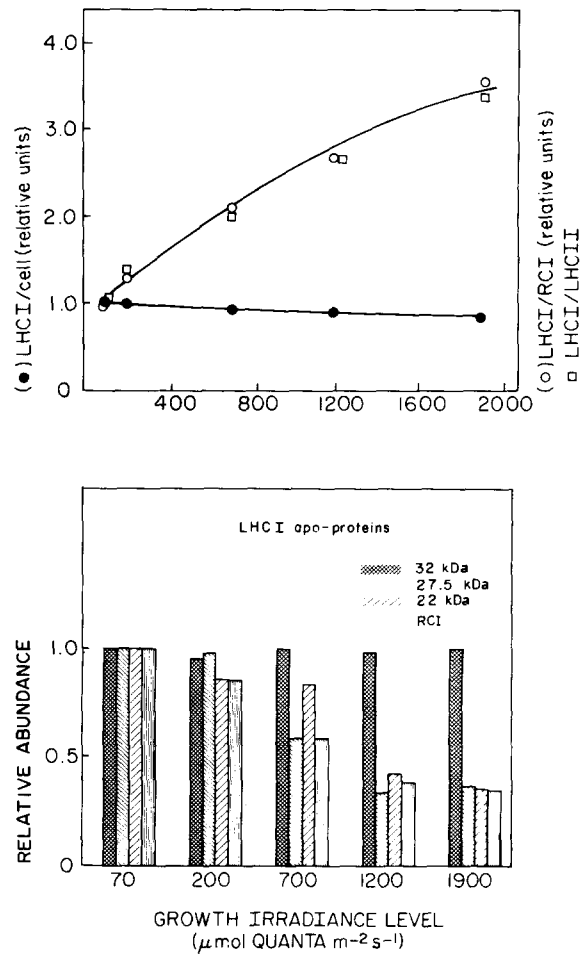

Fig. 3. Effect of growth irradiance on the relative cellular abundance of RC I, total LHC I, and total LHC II apoproteins (top) and the relative cellular abundance of RC I and individual LHC I apoproteins in *D. tertiolecta* (bottom). RC I/cell was determined from measurements of the Chl/P-700 ratios and Chl/cell. LHC I and LHC II were determined from densitometric scans of autoradiographs of Western blots. The relative abundance of individual RC I and LHC I apoproteins was calculated by normalizing the cellular level of each reaction center and of each apoprotein, respectively, measured at each growth irradiance level, to the level of the corresponding apoprotein measured in cells grown at 70 μmol quanta·m^{-1}·s^{-1}. The proportion of the individual apoproteins of the total LHC I for cells grown at 70 and 1900 μmol quanta·m^{-1}·s^{-1} is given in Table II.

number of LHC II apoproteins per RC II is relatively constant with irradiance, although the two methods give slightly different absolute numbers.

There are also large changes in the relative

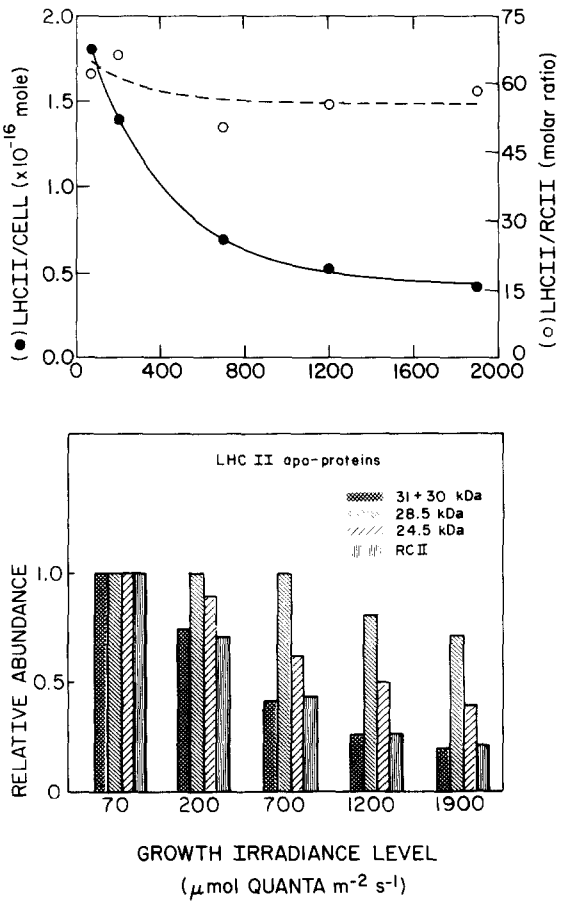

Fig. 4. Effect of growth irradiance on the cellular concentration of total LHC II apoprotein and the ratio of LHC II/RC II (top) and the relative cellular abundance of RC II and individual LHC II apoproteins in *D. tertiolecta* (bottom). RC II/cell was determined from oxygen flash yields and Chl/cell. LHC II was determined from densitometric scans of autoradiographs of Western blots. The relative abundance of individual RC II and LHC II apoproteins was calculated by normalizing the cellular level of each reaction center and of each apoprotein, respectively, measured at each growth irradiance level, to the level of the corresponding apoprotein measured in cells grown at 70 μmol quanta · $m^{-1} \cdot s^{-1}$. The proportion of the individual apoproteins of the total LHC II for cells grown at 70 and 1 900 μmol quanta · $m^{-1} \cdot s^{-1}$ is given in Table II.

concentrations of individual LHC II apoproteins. Between 70 and 1 900 μmol quanta · $m^{-2} \cdot s^{-1}$, the level of 28.5 kDa LHC II declined by less than 30%, while the levels of the 30 and 31 LHC II declined by 75%, in parallel with RC II.

In summary, in *D. tertiolecta* there are substantial changes in the relative abundance per cell of individual LHC I and LHC II apoproteins with increasing irradiance (70–1 900 μmol quanta · $m^{-2} \cdot s^{-1}$). Within both families, some apoproteins decline in concentration by more than 75%, while others decline by less than 30%. As RC I and RC II concentrations per cell also decline by about 75% with increasing irradiance, we conclude that some light-harvesting apoproteins are relatively more abundant per reaction center at HL than at LL. In this group are the 32 kDa apoprotein of LHC I and the 28.5 kDa apoprotein of LHC II.

Three of the four LHC II apoproteins, the 28.5, 30 and 31 kDa polypeptides, were clearly phosphorylated in vitro in membranes from LL cells (Fig. 2). Phosphorylation of the 24.5 kDa apoprotein was not detectable in membranes isolated from LL cells, but was detectable in membranes isolated from HL cells (data not shown).

The amino acid compositions of the ensemble of apoproteins which comprise LHC II from both HL- and LL-grown *D. tertiolecta* are presented in Table III, and are compared with similar analyses of LHC II from *Chlamydomonas reinhardtii* [30], *Arabidopsis thaliana* [31] and pea [32]. An analysis of the similarity of these amino acid compositions was performed according to a procedure of Cornish-Bowden [33], with LHC II from LL-grown *Dunaliella* as a reference. We opted for the procedure which assumes that the average length of the apoproteins in all the samples was constant. The results of this analysis indicate that the ensemble of LHC II apoproteins from LL and HL cells are not significantly different from each other, and are closely related to *Chlamydomonas*. Both *D. tertiolecta* and *C. reinhardtii* LHC II are significantly different from *Arabidobsis* and pea LHC II. A similar analysis shows that the pea and *Arabidopsis* apoproteins are as closely related to each other as the proteins from *Chamydomonas* and *Dunaliella*. The most marked differences in amino acid composition between the chlorophytes and the higher plants are due to the relative deficit of His, Ser and the relative enrichment of Gly and Arg in the algae compared with the other species.

Discussion

Our data establish that from many perspectives the pigment protein complexes of *D. tertiolecta*

TABLE III

AMINO ACID COMPOSITION (MOLAR FRACTION %) OF LHC II APOPROTEINS ISOLATED FROM HL AND LL GROWN *DUNALIELLA TERTIOLECTA* IN COMPARISON WITH *CHLAMYDOMONAS REINHARDTII* AND HIGHER PLANTS

Amino acid	*D. tertiolecta*		*C. reinhardtii*	Pea	*Arabidopsis thaliana*
	LL	HL			
Asp	9.4	8.2	9.3	10.7	11.0
Thr	3.8	3.8	5.3	4.0	3.9
Ser	3.8	3.8	3.8	7.7	7.9
Glu	9.8	9.0	9.6	9.2	7.1
Pro	8.2	10.2	7.6	8.0	6.3
Gly	19.0	13.9	13.2	14.7	13.0
Ala	7.4	8.6	11.9	10.4	13.0
Val	8.7	10.0	4.6	4.3	7.1
Met	1.3	1.7	1.8	1.2	2.7
Ile	3.1	3.3	4.6	1.8	3.1
Leu	8.8	9.6	12.2	10.4	9.8
Tyr	2.3	2.1	3.5	3.4	2.7
Phe	5.8	6.1	7.2	5.2	6.3
His	0.5	trace	1.6	0.9	1.2
Lys	4.7	4.3	5.6	5.2	1.9
Arg	3.5	3.1	3.4	2.7	1.9
Cornish–Bowden parameter	–	18.4	46.4	418.0	363.5

are similar to those found in other green algae and higher plants. The electrophoretic pattern of the pigment-protein complexes in LiDS gels found for *D. tertiolecta* is generally similar to those of higher plants and other green algae, including *Chlamydomonas reinhardtii* [34], *Euglena* [35] and *Bryoposis maxima* [36]. The LHC I apoproteins from *D. tertiolecta* (22, 27.5, 32 kDa) are somewhat larger than those reported for *Chlamydomonas*, where four LHC I apoproteins have been identified corresponding to 20, 24, 26 and 27 kDa [35]; LHC I apoproteins from higher plants tend to be even smaller (19–23 kDa) [1,21,23,29]. The molecular masses of the LHC II apoproteins from *D. tertiolecta* (24.5, 28.5, 30, 31 kDa) are similar to those reported for *Chlamydomonas*, which has three major apoproteins with molecular masses of 29, 25 and 24 kDa. Furthermore, the amino acid compositions of the complexes for the two species are similar (Table III). Finally, the LHC II apoproteins from *D. tertiolecta* all undergo phosphorylation, implying that the phosphorylation site of the individual apoproteins is conserved in the marine chlorophyte.

In spite of the apparent similarities between the pigment-proteins of *Dunaliella* and *Chlamydomonas*, there is at least one important difference, namely, the pigment composition of LHC II. LHC II of *Chlamydomonas* reportedly has a 'classical' Chl *a*/Chl *b* ratio of 1.1–1.2 [30], and is invariant. In *D. tertiolecta* the Chl *a*/Chl *b* ratio of LHC II is higher and varies from about 2.4 to 4.7 as cells adapt to different growth irradiance

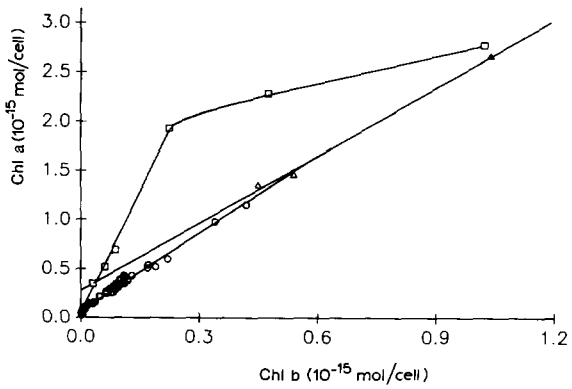

Fig. 5. Relationship between cellular levels of Chl *a* and Chl *b* in green algae grown at various irradiances. Data related to *D. tertiolecta* (□), *Chlamydomonas reinhardtii* (△), and *Chlorella vulgaris* (○).

levels. This difference in behavior of LHC II has a major impact on the cellular Chl *a* and Chl *b* contents. In Fig. 5 we present the relationship between cellular Chl *a* and Chl *b* contents for *D. tertiolecta* [11], *C. reinhardtii* [38], and *Chlorella vulgaris* [39]. The individual points on each line reflect different growth irradiances under conditions of nutrient abundance. The slope of the line for *D. tertiolecta* has a maximum value of 5.75, and decreases with increasing cellular Chl (decreasing growth irradiance). In contrast, the slope for *Chlorella* is 2.63 and for *Chlamydomonas* is 2.28 and appears to be constant. Since LHC II accounts for the vast majority of cellular Chl *b* in *C. reinhardtii* and *C. vulgaris*, there are two ways of explaining the steep slope in *D. tertiolecta*: either (a) this alga contains very little LHC II, or (b) its LHC II displays an unusually high Chl *a*/Chl *b* ratio. Our previous analysis of LHC II from HL and LL cells of *D. tertiolecta* [14] established that it is the second alternative which obtains. Moreover, the non-linearity of the relationship between Chl *a* and Chl *b* for *D. tertiolecta* is consistent with the reported changes in the Chl *a*/Chl *b* ratio of LHC II as a function of growth irradiance. Thus, application of this simple pigment analysis to other green algae should reveal not only which species may contain unusual forms of LHC II but what growth irradiances are required to elicit the unusual forms. In the case of *D. tertiolecta*, irradiances around 30 μmol quanta $\cdot$ $m^{-2} \cdot s^{-1}$ are required for the accumulation of approximately 'normal' LHC II, while irradiances of 1 200–1 900 $\mu mol \cdot m^{-2} \cdot s^{-1}$ permit enrichment for the unusual LHC II. In HL cells the average pigment composition per LHC II apoprotein is 5 Chl *a*, 1 Chl *b* and two lutein molecules, while at LL the average pigment composition per LHC II apoprotein is 5 Chl *a*, 2 Chl *b* and two lutein molecules [14]. Our present results indicate that as Chl *a*/Chl *b* ratios change within LHC II there are corresponding changes in abundance of individual apoproteins. We propose that all LHC II apoproteins of *D. tertiolecta* bind five molecules of Chl *a*, two molecules of lutein, but differ in their capacity for binding Chl *b*. Presumably, the 28.5 kDa apoprotein, which predominates at HL (Fig. 4), binds fewer Chl *b* molecules than the 30 and 31 kDa apoproteins that predominate at LL.

To establish firmly that the apoproteins of LHC II differ in their capacity to bind Chl *b*, it will be necessary to separate individual monomeric LHC II complexes. Whether this will be possible for the LHC II of *D. tertiolecta* remains to be seen. An alternative approach would be to clone the corresponding LHC II genes of *Dunaliella* in bacteria and use the in vitro LHC II reconstitution technique [40] to assess the pigment-binding properties of each apoprotein. Sequencing of such clones would also provide insight into the modes of binding of Chl's and accessory pigments to apoproteins.

Studies with bacterial pigment-protein complexes have implicated histidine in the attachment of bacteriochlorophyll to apoproteins [41,42]. Since LHC II complexes from higher plants contain at least three Chl *a* and three Chl *b* molecules per apoprotein [1] it might be expected that the apoprotein would contain at least six histidines. However, higher plant *Cab* genes generally code for proteins containing only three histidines [9,31]. Our amino acid analyses indicate that LHC II from *D. tertiolecta*, and especially LHC II from HL cells, contain fewer histidines and carry fewer Chl *b* molecules than higher plant LHC II's. It may be therefore that only Chl *b* is attached to LHC II apoproteins by histidine, with Chl *a* attached via a different mechanism. We do not suggest that Chl *a* can never be attached to apoproteins via histidine; for example, the apoproteins of RI I are thought to bind Chl *a* molecules via their numerous hsitidine residues [43].

The physiological significance of the changes in pigment composition of HL and LL cells of *D. tertiolecta* is a key question. Changes in the ratios of LHC I/LHC II with growth irradiance appear to occur in this species (Figs. 3 and 4) in spite of a relatively constant RC I/RC II ratio [13]. These results suggest that as cells adapt to higher irradiances relatively more photons are absorbed by PS I, and might contribute to an increase in the relative proportion of cyclic phosphorylation. Changes in the pigmentation of LHC II itself, however, may have an additional function. We have previously shown [14] that the transfer of excitation energy from Chl *b* to Chl *a* in purified LHC II preparations from LL cells is higher than the transfer of excitation energy from lutein to

Chl *a* in LHC II from HL cells. While lutein transfers excitation energy to Chl *a*, the efficiency of transfer is much less than observed for Chl *b* [14,44]. Lutein is likely to prevent photooxidative damage to PS II Chl molecules both by 'screening' the core of PS II from excess excitation and by quenching triplets [44,45].

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