Enrichment of the Light-Harvesting Complex in Diadinoxanthin and Implications for the Nonphotochemical Fluorescence Quenching in Diatoms[†]

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ABSTRACT: The pigment composition of diatoms differs from that of green algae and plants. Diatoms contain chlorophyll (Chl[1]) *c*, fucoxanthin, and diadinoxanthin (DD). An intermittent light regime during growth induced a large increase in the DD content in the marine planktonic diatom *Phaeodactylum tricornutum*. Light-harvesting complex containing fucoxanthin (LHCF) subunits were purified on a sucrose gradient after treatment of thylakoid membranes with a mild detergent. DD was found in all the LHCF fractions: a "major" composite LHCF fraction and the two fractions where some LHCF was associated with photosystem centers. For cells enriched in DD, most of the additional DD molecules were bound to the major LHCF fraction. The DD enrichment of the major LHCF fraction was accompanied by a decrease in the fucoxanthin to Chl *a* ratio. Either some fucoxanthin molecules were replaced by DD or there could be a relative enrichment of subunits rich in DD at the expense of fucoxanthin/Chl *c* rich subunits. Under high light illumination, a higher degree of de-epoxidation of DD into DT was observed for the major LHCF of cells enriched in DD. This fraction has the higher DD content and the higher degree of de-epoxidation. These results show that the distal antennae, probably mostly isolated as the major LHCF fraction, play a crucial role in the formation of NPQ, its amplitude depending on the amount of DD bound and on the degree of de-epoxidation (Lavaud et al. (2002) *Plant Physiol. 129,* 1398−1406).

Diatoms are unicellular photosynthetic eukariots that play a key role in ocean carbon, silica, and azote cycles (*1, 2*). They are ubiquitous and the major group of phytoplanktonic marine algae. They experience large fluctuations in light intensity due to unpredictable water motions that can vary over several orders of magnitude (*3*−*5*). As other photosynthetic organisms (*6, 7*), they have developed efficient photoprotective mechanisms (*8*−*13*) in order to minimize photoinhibition that could result from their periodic exposure to excess light intensities when transported to the water column surface (*14*). Among the short-term "safety valves" that are activated by a sudden increase in light intensity, the photoprotective nonradiative dissipation of the light energy absorbed in excess is an important mechanism (*15*). This phenomenon is attributed to rapid modifications within the light-harvesting complexes (LHC)[1] of photosystem II (PS II) leading to a decrease in the excitation pressure on PS II (*16, 17*). It is designated as a nonphotochemical fluorescence quenching (NPQ) process and measured by a decrease in chlorophyll (Chl) fluorescence intensity under high light. In higher plants and green algae, NPQ is controlled by the formation of a proton gradient across the thylakoid membrane and by reversible conversion of epoxidized (violaxanthin) to de-epoxidized (antheraxanthin and zeaxanthin) forms of xanthophylls (the so-called xanthophyll cycle; for reviews, see refs *16*−*20*). In diatoms, the xanthophyll cycle consists of the conversion of the monoepoxide diadinoxanthin (DD) into the de-epoxide diatoxanthin (DT) under excess light and vice versa under limiting light or darkness (*21*). The formation of DT is associated with the onset of NPQ within the light-harvesting complexes (LHCF) (*9, 11, 12, 22*). In higher plants, the location and cycling of xanthophylls within LHC subunits have been the subject of many investigations (see refs *23*−*25* and citations within). It has been shown recently that in higher plants, a specific LHC subunit, the CP22 (PsbS), binds zeaxanthin in vitro (*26*). It plays a crucial role in NPQ. The *Arabidopsis* psbS deletion mutant is not capable of NPQ (*27*), and there is a stoichiometric dependence of NPQ capacity on the amount of PsbS (*28, 29*).

There is a lack of knowledge concerning the exact nature and organization of LHCF subunits in diatoms and especially the location of DD and DT in the antenna complex. The whole organization of the photosynthetic apparatus in diatoms differs in many aspects from that of green plants: (i) their pigment composition is different, with Chl *c* and fucoxanthin as the main xanthophyll; (ii) the xanthophyll concentration relative to Chl can be 2−4 times higher than in higher plants (*30, 31*); (iii) there is no grana stacking and no segregation of PS II and PS I (*32*). LHCF subunits are made of several highly homologous proteins encoded by a multigene family (fucoxanthin chlorophyll proteins, FCP

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[1] Abbreviations: Chl, chlorophyll; CL, continuous light regime (16 h light/8 h dark per day); DD, diadinoxanthin; DT, diatoxanthin; IL, intermittent light regime (5 min of light per hour); LHC, light-harvesting complex; LHCF, light-harvesting complex containing fucoxanthin; NPQ, nonphotochemical chlorophyll fluorescence quenching; PS II and I, photosystems II and I.

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(*33*)). No obvious orthologues of some of the important components of LHC of higher plants PS II (e.g., the minor antennae CP 26, CP 29, and PsbS) have been found in *Phaeodactylum tricornutum* Böhlin or within the genome sequence of *Thalassiosira pseudonana* Hustedt (Hasle and Heimdal) (*17*); C. Bowler, personal communication).

It is known that the DD content of diatoms can be modulated by the light regime during culturing (*34*−*36*). We used an intermittent light regime to grow the marine planktonic diatom *P. tricornutum*. As a result, there was a specific increase in the DD content of cells. The additional DD was convertible to DT under excess light exposure of whole cells, and concomitantly, photoprotective energy dissipation was increased (*12*).

In the present study, our goal was to locate the DD molecules and determine their degree of conversion into DT within the different pigment−protein complexes. We present the pigment content and spectroscopic properties of LHCF fractions isolated from *P. tricornutum* cells grown under an intermittent light regime and compare them with those of cells grown under a "classical" light regime, which have a smaller DD content. The data obtained provide a possible explanation for the larger NPQ found in cells enriched in DD (*12*).

MATERIALS AND METHODS

Cultures. Phaeodactylum tricornutum Böhlin (from the algal culture collection of Laboratoire Arago, Banyuls, France) cells were grown photoautotrophically in sterile natural seawater F/2 medium (*37*). Cultures of 300 mL were incubated at 18 °C in airlifts continuously flushed with sterile air. They were illuminated at a light intensity of 40 μmol of photons m^{-2} s^{-1} with white fluorescent tubes (Claude, Blanc Industrie, France) with a 16 h light/8 h dark cycle for continuous light (CL) cells or with a 5 min light/55 min dark cycle for intermittent light (IL) cells. The growth rate under IL illumination was reduced from 0.65 to 0.065 day^{-1} partly because of the 8-fold lower total amount of light supplied. CL cells were collected after 3−4 days, and IL cells were collected between 30 and 40 days after dilution. Part of the culture was then diluted in fresh medium to maintain cells in the exponential growth phase.

Pigment Content. Pigment analyses were performed by HPLC as described previously (*11*). Cells were filtered onto a filter paper (Millipore AP-20 prefilter) rapidly frozen into liquid nitrogen. After thawing, the filter was placed in a methanol/acetone solution (70:30, v/v) to extract the pigments. For the light-harvesting complexes isolated on the sucrose gradient, pigments were extracted by a phase separation procedure. Samples were first mixed with a methanol/acetone (50:50, 1 vol) solution completed with ether (1 vol) and water/NaCl 10% (2 vol). Published extinction coefficients for chlorophylls (*38*) and for diadinoxanthin (DD) and diatoxanthin (DT) (*39*) were used. Cell counts were performed with a Thoma hematocymeter, using the public domain NIH Image program (U.S. National Institutes of Health).

Spectroscopy. The 77 K absorption spectra were obtained with a DW-2 Aminco spectrophotometer equipped with a homemade Dewar holder. The 77 K fluorescence excitation spectra were measured with a F-4500 Hitachi spectrophotometer. The half-bandwidth for emission and excitation was 2.5 nm. Samples were dark-adapted and then concentrated on a Millipore AP-20 prefilter that was immediately frozen in liquid nitrogen before measurement.

Isolation of Light-Harvesting Complexes. The light-harvesting complexes subunits were isolated as described (*31*). Chloroplasts were extracted and suspended in a 50 mM Hepes/KOH buffer (pH 7.6) with 5 mM $MgCl_2$, 2 mM $MnCl_2$, 10 mM KCl, 6 mM Na_2/EDTA, 5 mM aminocaproic acid, 0.6 M sorbitol, and protease inhibitors (1 mM phenylmethanesulfonyl fluoride and 1 mM benzamidine). They were disrupted in a French pressure cell and then resuspended in the same buffer, without sorbitol, in the presence of digitonin at a detergent/Chl *a* ratio of 80:1 and incubated for 1 h at 4 °C. The homogenate was loaded on top of a sucrose gradient (10−55%) and centrifuged at 14000*g* for 15 h at 4 °C. The three bands obtained corresponded, from the bottom to the top of the gradient, to PS I and PS II enriched fractions, with some LHCF remaining bound, and the major free LHCF band (see Results for a detailed description). For preilluminated cells, epoxidation of DT into DD was prevented by keeping all fractions on ice and by a faster handling of the samples.

RESULTS

Ultrastructure, Photosynthetic Parameters, and Pigment Content of the CL and IL Cells. Phaeodactylum tricornutum Böhlin cells were grown at low light intensity (40 μmol of photons m^{-2} s^{-1}) under two different light regimes: a 16 h light/8 h dark cycle for "continuous light" (CL) cultures and a 5 min light/55 min dark cycle for "intermittent light" (IL) cultures. Cell size and chloroplast ultrastructure, as illustrated by the number of thylakoids and their organization, were similar under the two light regimes. The photosynthetic parameters of both types of cells have been previously described (*12*). They were all similar, especially photosystem stoichiometries, PS II antenna size, and the oxygen emission versus light intensity curve. The larger change in the pigment content of whole cells was that of DD, which was more than twice in IL cells (Table 1). Fucoxanthin and Chl *c* were decreased to a much lesser extent. At the low light intensity used for culturing, no DT was present before exposure of the cells to excess light (Table 1). DD absorbed in the blue region of the visible spectrum with a peak at 495 nm clearly visible both in the absorption spectra of cells enriched in DD (Figure 1A, continuous line) and in the difference spectrum of the absorption spectra of cells with different DD content (Figure 1A, bottom part). In the 77 K fluorescence excitation spectra of the fluorescence emission at 687 nm (characteristic of PS II), the contributions of Chl *a*, Chl *c*, and fucoxanthin to the transfer of excitation energy were visible (Figure 1B). The contribution of DD was less marked showing that all DD molecules, and specifically the additional DD molecules in IL cells (Figure 1B, arrow), would thus not be able to transmit their excitation energy to Chl *a*.

Pigment Content of the Light-Harvesting Complexes of Dark-Adapted CL and IL Cells. After disruption of the chloroplasts of dark-adapted cells in the presence of mild detergent, the homogenate was loaded onto a sucrose gradient and centrifuged. Three LHCF bands were obtained (Figure 2A). The band at the top of the gradient (a brown band, at

Table 1: Pigment Composition of Dark-Adapted CL and IL Cells Grown under an Intensity of 40 μmol of Photons m^{-2} s^{-1} and of Corresponding Sucrose Gradient Fractions Containing Light-Harvesting Complexes (LHCF) and Photosystems (PS)

	pigment composition,[a] mol/(100 mol of Chl *a*)				
pigments	CL cells	major LHCF	PS II + LHCF	PS I + LHCF	pellets
Chl *c*	15.9 ± 0.3	24.8 ± 1.0	8.7 ± 0.06	5.8 ± 1.0	8.4 ± 1.8
fucoxanthin	68.4 ± 3.0	110.0 ± 5.9	47.3 ± 3.2	31.0 ± 1.5	52.0 ± 3.4
DD	9.5 ± 1.5	9.6 ± 2.3	8.9 ± 0.4	10.5 ± 0.7	6.7 ± 1.0
DT	0	0	0	0	0
β-carotene	8.0 ± 1.1	1.8 ± 0.2	12.3 ± 2.7	15.1 ± 1.8	8.6 ± 1.9
	pigment composition,[a] mol/(100 mol of Chl *a*)				
pigments	IL cells	major LHCF	PS II + LHCF	PS I + LHCF	pellets
Chl *c*	13.4 ± 0.4	19.9 ± 1.2	8.0 ± 2.4	5.1 ± 0.4	8.7 ± 2.4
fucoxanthin	65.5 ± 3.5	95.6 ± 3.3	46.0 ± 3.3	30.0 ± 3.6	51.4 ± 0.2
DD	21.6 ± 1.8	27.6 ± 3.1	13.4 ± 1.2	13.2 ± 1.7	8.6 ± 0.2
DT	0	0	0	0	0
β-carotene	8.4 ± 0.2	2.3 ± 0.7	12.0 ± 1.4	14.3 ± 2.2	8.3 ± 1.6

[a] Total Chl *a* per cell was 0.38 ± 0.05 pg for CL cells and 0.35 ± 0.05 pg for IL cells. The data (±SD) are the average of three to five independent measurements.

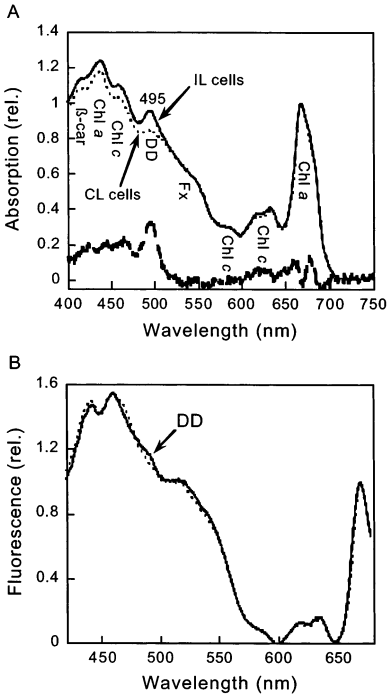

FIGURE 1: (A) 77 K absorption spectra of CL (dotted line) and IL (continuous line) cells. The main pigment contributing to each band (Chl, chlorophyll; β-car, β-carotene; DD, diadinoxanthin; Fx, fucoxanthin) and the absorption peak of DD (495 nm) are indicated. The difference spectrum of IL cells minus CL cells is the trace at the bottom part of the panel (it was multiplied by 3). (B) 77 K excitation spectra of the fluorescence emission at 687 nm. The arrow indicates the excitation peak of DD. All spectra are normalized to 667 nm; the minimum value at 600 nm is taken as the origin of the fluorescence spectra.

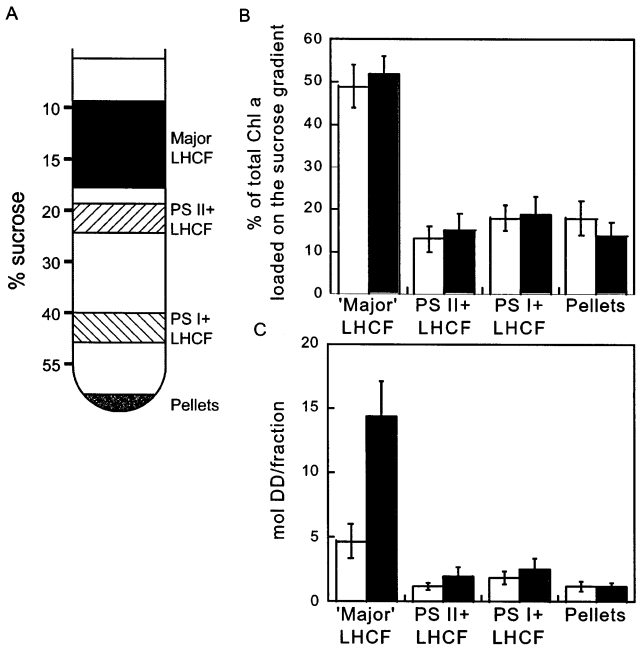

FIGURE 2: (A) Distribution of the three LHCF bands on the sucrose gradient after solubilization of the thylakoids membranes by a mild detergent and centrifugation. (B) Repartition of the total Chl *a* loaded onto the gradient. (C) Comparison of the amount of DD present in the different bands: CL (white boxes) and IL (black boxes) cells. Data (±SD) are the average of three to four independent measurements.

10−15% sucrose) contained 50% of the loaded Chl *a* (Figure 2B) and the majority of DD (Figure 2C). The two other heavier bands contained 15−20% Chl *a* each. This distribution is similar to what was observed when the same purification procedure was used with brown algae that belong to the same phylum (Heterokontophyts) (*40, 41*). The three bands obtained in *P. tricornutum* can be identified as follows. The top band corresponds to a band enriched in LHCF designated as the "major" LHCF (Figure 2A). In brown algae, it is quasi devoid of photosystems reaction centers (*31, 42*). According to refs *38* and *43*, the two other bands are enriched in photosystems with their respective LHCF. In the present study, the intermediate band at 20% sucrose is enriched in PS II and the bottom band is enriched in PS I (Figure 2A). Pellets represented 15−20% of the total loaded Chl *a* (Figure 2B), and their pigment content was similar for both types of cells as shown in Table 1. This similarity in the pigment content of the pellets is important for valuable comparison of LHCF subunit pigment content of CL and IL cells.

It is known that the core complexes of the two photosystems contain only Chl *a* and β-carotene. Therefore, in proportion to Chl *a*, Chl *c* and fucoxanthin were more abundant in the major LHCF fraction than in the fractions enriched in PS II and PS I core complexes (Table 1). The situation was reversed for β-carotene, which is more

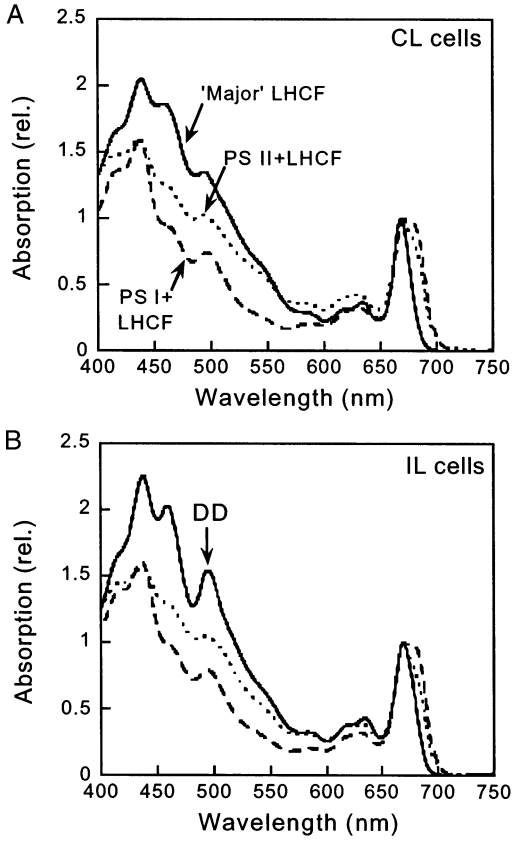

FIGURE 3: 77 K absorption spectra of the three LHCF fractions isolated on sucrose gradient for the two types of cells: (A) CL cells; (B) IL cells. The "major" LHCF (continuous line) and the two LHCF associated with PS II (dotted line) and PS I (dashed line) are shown. All spectra are normalized to 667 nm.

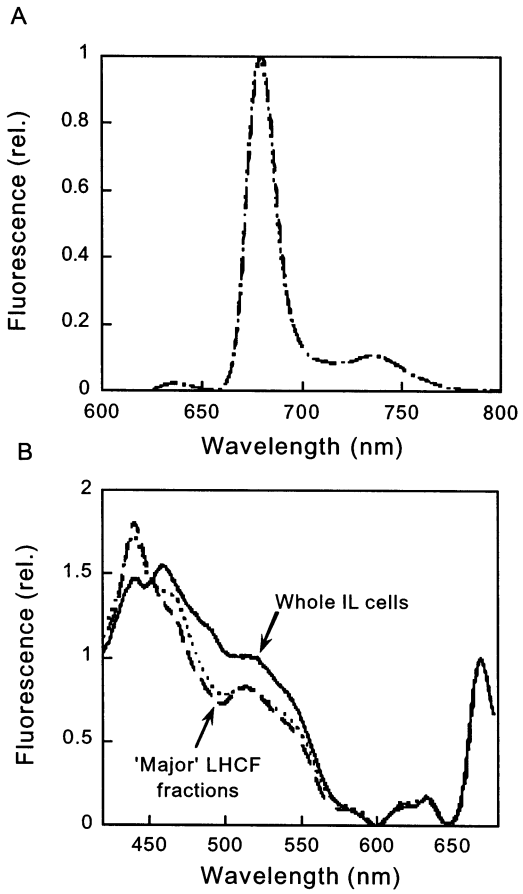

FIGURE 4: (A) 77 K emission fluorescence spectrum of Chl *a* excited at 440 nm of the major LHCF fraction of CL cells (dotted line) and IL cells (dashed line). Spectra are normalized to 678 nm. (B) 77 K excitation spectra of Chl *a* emission at 678 nm of whole IL cells (continuous line) and the "major" LHCF fraction of CL cells (dotted line) and IL cells (dashed line). Spectra are normalized to 667 nm. The minimum value at 600 nm is taken as the origin.

abundant in the fractions enriched in photosystems. The traces of β-carotene in the major LHCF were indicative of a small contamination as already reported for isolation of LCH-II in higher plants (*24*). From a comparison of the DD/Chl *a* in whole cells and in different fractions for IL and CL cells, it is obvious that the main enrichment in DD, when the DD pool was increased (IL cells), was in the major LHCF fraction (Table 1, Figure 2C).

Spectroscopic Properties of the Light-Harvesting Complexes of Dark-Adapted CL and IL Cells. The 77 K absorption spectra of the major LHCF and the two photosystems fractions in the two types of cells are shown Figure 3. The spectra of the major LHCF fractions differed. The enrichment in DD of the major LHCF fraction of IL cells was clearly visible by the increased 495 nm peak (Figure 3B, arrow). The spectra of PS II and PS I fractions of both types of cells were quite similar. They showed a smaller absorption in the blue region, compared to the major LHCF fraction, indicative of a decrease in Chl *c*, fucoxanthin, and DD content. The two photosystem fractions also have a larger concentration in long-wavelength Chl *a* than the major LHCF fractions.

The 77 K fluorescence emission of the major LHCF of CL and IL cells was maximal at 678 nm with a secondary peak at 735 nm (Figure 4A). The small fluorescence at 635 nm showed that the majority of Chl *c* molecules still transferred excitation energy to Chl *a* and were not fluorescing. In this context, it is noticeable that the difference already observed in the Chl *c* content in the major LHCF of the two types of cells was not due to an increase in free pigments in IL cells but reflected the difference observed between whole cells (Table 1). When comparing the 77 K excitation spectra of the emission at 678 nm (maximum emission of the major LHCF fractions) of whole cells and the major LHCF of the two types of cells, one can see a large band in the 500−550 nm range in the LHCF fractions, which showed that most of the fucoxanthin molecules remained correctly bound and transferred their excitation energy to Chl *a* (Figure 4B). It was not the case for the DD molecules whose contribution around 495 nm was no longer detectable especially for IL cells (compare Figure 1B and Figure 4B). However, the fact that the DD absorption peak was not blue-shifted after purification of LHCF (Figure 3) is a possible indication that DD remained connected to complexes, as already reported for the analogue violaxanthin (*44*), but probably not in a configuration that still allowed energy transfer to Chl *a* as observed for whole cells (Figure 1B). Hence, considering that free pigments are a minority (no free pigments were visible at the top of the sucrose gradient; Figure 2A), one can further analyze the pigment content of LHCF fractions (Table 2). The pigment content of the two major LHCF fractions was different. There was significantly less fucoxanthin and Chl *c* but more DD in the IL fraction. However, the xanthophyll (fucoxanthin + DD) to Chl *a* ratio as well as the ratio Chl *c*/fucoxanthin remained constant.

Table 2: Pigment Composition of the Major LHCF Fractions of CL and IL Cells

	pigment composition,[a] mol/(100 mol Chl *a*)	
pigments	CL cells	IL cells
fucoxanthin	110.0 ± 5.9[b]	95.6 ± 3.3[b]
DD	27.6 ± 3.1	9.6 ± 2.3
Chl *c*	24.8 ± 1.0[b]	19.9 ± 1.2[b]
DD + fucoxanthin	119.6 ± 6.0[c]	123.2 ± 7.3[c]
Chl *c*/fucoxanthin	0.225	0.208

[a] Data (±SD) are the average of three to five independent measurements. [b] From the Student test (statistical test for mean values comparison), the mean values are significantly different ($p < 0.05$). [c] From the Student test, the mean values are not significantly different ($p < 0.05$).

Pigment Content and Spectroscopic Properties of the Light-Harvesting Complexes of Illuminated IL Cells. With the low light intensity used for culturing, no DT was present in whole cells (Table 1). When cells were shifted to excess light, the de-epoxidation of DD into DT took place (*22*). We have previously shown that under excess light, the degree of de-epoxidation was higher in IL cells, resulting in a larger accumulation of DT (*22*). For a light intensity of 2000 μmol of photons m^{-2} s^{-1} during 1 h, the amount of DT formed was 5 and 12.5 mol of DT/100 mol of Chl *a* in CL and IL cells, respectively. It corresponded to a de-epoxidation degree of 50% and 65%, respectively (*12*).

To determine the degree of de-epoxidation in the three LHCF fractions in IL cells, they were strongly illuminated for 20 min prior to purification of the fractions. When comparing the pigment content of dark-adapted and illuminated cells, one can observe that during illumination the amount of all pigments remained constant with a small increase in the sum DD + DT due to a slight de novo synthesis of DT (*9*). While the major LHCF fraction showed a degree of de-epoxidation similar to whole cells, the degree was clearly less in the two photosystems fractions (Table 2). The amount of DT reached in the major LHCF fraction was 3 times that reached in the PS + LHCF fractions. The conversion of DD into DT was illustrated by peaks at 438, 474, and 508 nm in the difference absorption spectrum of the major LHCF of illuminated cells minus dark-adapted cells (Figure 5). The 508 nm peak has already been assigned to DT (*45*). The three bands are very similar to those observed in the zeaxanthin-minus-violaxanthin difference spectrum in higher plants (*46*).

DISCUSSION

Additional DD Binds to the "Major" Light-Harvesting Complex in DD-Enriched Cells. In *P. tricornutum*, the size of the DD pool increases under intermittent light and a larger fraction of the pool is susceptible to de-epoxidation (*12, 22*). It was important to determine the exact location of the additional DD molecules in the IL cells. Indeed, the corresponding DT molecules produced under high light were shown to enhance the dissipation of excess energy and were therefore likely to be bound to the antenna subunits responsible for the NPQ process (*12*).

In brown algae that belong to the same phylum as diatoms, it has been shown that an LHCF monomer can contain seven Chl *a* and as many fucoxanthin molecules (*41, 42*). Nothing is known about the number of violaxanthin (the analogue of DD in brown algae) bound per subunit. After solubilization of the *P. tricornutum* thylakoids membranes by a mild detergent and fractionation of the pigment−protein complexes, all the fractions containing LHCF antenna are found to contain DD as reported for its violaxanthin analogue in higher plants (*24, 47*). In IL cells, compared to CL cells, nearly all of the additional DD molecules are bound to the major composite LHCF fraction with only a minor enrichment for the LHCF fractions that remain associated with the photosystem centers. Two hypotheses can be proposed to explain the quasi specific DD enrichment of the major composite LHCF fraction in IL cells. The first could be a pigment−pitment "replacement". If we assume that the Chl *a* stoichiometry of the LHCF subunit in diatoms is the same as in brown algae, we can estimate that in IL cells one subunit would bind two DD molecules. In CL cells, only one DD would be bound per LHCF subunit. Such a doubling of the DD amount bound to one LHCF subunit would be explained by a replacement of some fucoxanthin molecules by DD. This is supported by the observation that the enrichment in DD in IL cells is correlated with a stoichiometric parallel decrease in fucoxanthin (Table 3). Such a "competition" of two xanthophylls of related structures, as is the case for DD and fucoxanthin (*48, 49*), for a common binding site is conceivable. In higher plants, the possibility

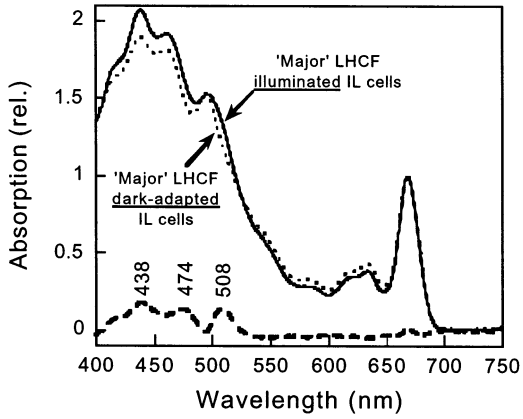

FIGURE 5: The 77 K absorption of the "major" LHCF fraction of dark-adapted (dotted line) and illuminated (continuous line) IL cells. Spectra are normalized to 667 nm. Illumination at 5300 μmol of photons m^{-2} s^{-1} for 20 min induced a 67% de-epoxidation of DD into DT (see Table 3). Three absorption bands (438, 475, and 508 nm) due to the conversion of DD in DT during light exposure are visible in the difference spectrum (illuminated cells minus dark-adapted cells, bottom dashed line).

Table 3: Pigment Composition of Illuminated IL Cells (20 min, 5300 μmol of Photons m^{-2} s^{-1}) and of Corresponding Sucrose Gradient Fractions Containing Light-Harvesting Complexes (LHCF) and Photosystems (PS)

	pigment composition, mol/(100 mol Chl *a*)				
pigments	IL cells, dark-adapted	IL cells, illuminated	major LHCF	PS II + LHCF	PS I + LHCF
Chl *c*	13.0	13.2	18.2	8.9	4.4
fucoxanthin	68.0	66.7	93.2	55.6	27.2
DD	20.7	9.1	9.3	7.7	10
DT	0	13.9	19.1	6.0	6.3
β-carotene	8.5	8.4	2.1	10.6	15.4
DD + DT	20.7	23.0	28.4	13.7	16.3
DED[a]	0	60	67	44	39

[a] DED: de-epoxidation degree (in %): [DT/(DD + DT)] × 100.

that a given site of a LHC protein can bind different xanthophylls has been demonstrated both in vitro (*20*) and in vivo (*25, 50, 51*). However, the existence of LHCF subunits with different pigment content is very likely. Since, in parallel to the increase in DD content, Chl *c* decreases to the same extent as fucoxanthin, some subunits could be specifically rich in DD while others could mainly bind fucoxanthin and Chl *c*. The relative abundance of the different subunits would be modulated by light. It has already been shown that the expression of the different *lhcf* genes is light-dependent (*52, 53*). The regulation of the LHCF protein synthesis has been studied as a function of light intensity (*54*−*57*) but not as a function of the light period. In IL cells, the amount of subunits rich in DD would be increased and that of subunits rich in fucoxanthin and Chl *c* would be decreased compared to the situation in CL cells.

Fucoxanthin, Chl *c*, and Chl *a* are the main contributors to the energy harvesting (Figure 1A) and transfer to the photosystem reaction centers (Figure 1B). Therefore, the modulation in DD content allows the cells to regulate their capacity for dissipating energy under excess light without changing significantly the light harvesting under limiting conditions.

Xanthophyll Cycle within Light-Harvesting Complexes in DD-Enriched Cells. Under excess light, a higher degree of de-epoxidation occurs in DD enriched cells (*12, 22*). To be de-epoxidized, xanthophylls have to be accessible to the de-epoxidase that is localized in the lipid matrix (*19*). The fraction of DD that can be transformed to DT is thus likely located at the periphery of the pigment−protein complexes (*25*). The fact that DD is more abundant and de-epoxidized to a larger extent within the major LHCF of IL cells leads to the proposal of a peripheral location for the additional DD molecules. Comparison of absorption and excitation spectra (Figure 1) shows that some of the additional DD molecules interact with Chl *a* with low efficiency. This could also suggest a peripheral location for these molecules. In brown algae, among the eight fucoxanthin molecules which can bind to one LHCF monomer, two are proposed to have a role in the stabilization of the LHCF (*41, 58*) by analogy with the two central lutein molecules in the LCH-II of higher plants (*59*). The binding sites of the six other molecules of fucoxanthin remain unknown. In the framework of our "DD−fucoxanthin replacement hypothesis" and if we assume the same fucoxanthin stoichiometry per monomer in *P. tricornutum*, at least two fucoxanthin molecules could be bound at the periphery of the LHCF. Under intermittent light, these molecules would likely be replaced by DD and then be easily accessible to the de-epoxidase.

In a previous report, we have shown that NPQ is enhanced in IL grown cells and linearly related to the amount of DT formed upon light exposure (*12*). Our present results show that the majority of the additional DD molecules are bound to the major LHCF and that the largest degree of de-epoxidation is observed in this fraction. It indicates that NPQ mostly takes place in the distal antennae, essentially isolated as the major LHCF fraction. Similar conclusions were recently reached for higher plants (*44, 60*). Considering the most recent results on the understanding of the NPQ process in higher plants and the essential role of PsbS and the fact that it can bind zeaxanthin (the analogue of DT) (*26, 29*), one can conclude that the orthologue of PsbS in diatoms (if it exists) is likely to be found in the composite major LHCF fraction.

CONCLUDING REMARKS

By regulation of the light-harvesting through a fine-tuning of their xanthophyll content, diatoms are well suited to avoid the serious damages that may result from light stresses without any significant decrease in their capacity for light collection under light-limiting conditions. Because planktonic diatoms can modulate their DD pool size in the field (*61*−*63*), this ability likely explains their successful adaptation to the fluctuating light environment (*3, 4*).

Because DD de-epoxidation into DT can be readily observed by absorption changes (see Figure 6), these changes will be used for further studies on the regulation of energy dissipation mechanisms within the light-harvesting complexes. Such measurements have previously been performed with higher plants (*64, 65*). Intermittent light-grown diatoms being enriched in DD provide a unique model for further studies on photoprotective energy dissipation process.

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