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REGULAR PAPER

Structurally flexible macro-organization of the pigment–protein complexes of the diatom *Phaeodactylum tricornutum*

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Abstract By means of circular dichroism (CD) spectroscopy, we have characterized the organization of the photosynthetic complexes of the diatom *Phaeodactylum tricornutum* at different levels of structural complexity: in intact cells, isolated thylakoid membranes and purified fucoxanthin chlorophyll protein (FCP) complexes. We found that the CD spectrum of whole cells was dominated by a large band at (+)698 nm, accompanied by a long tail from differential scattering, features typical for psi-type (polymerization or salt-induced) CD. The CD spectrum additionally contained intense (–)679 nm, (+)445 nm and (–)470 nm bands, which were also present in isolated thylakoid membranes and FCPs. While the latter two bands were evidently produced by excitonic interactions, the nature of the (–)679 nm band remained unclear. Electrochromic absorbance changes also revealed the existence of a CD-silent long-wavelength (~545 nm) absorbing fucoxanthin molecule with very high sensitivity to the transmembrane electrical field. In intact cells the main CD band at (+)698 nm appeared to be associated with the multilamellar organization of the thylakoid membranes. It was sensitive to the osmotic pressure and was selectively diminished at elevated temperatures and was capable of undergoing light-induced reversible changes. In isolated thylakoid membranes, the psi-type CD band, which was lost during the isolation procedure, could be partially restored by addition of Mg-ions, along with the maximum quantum yield and the non-photochemical quenching of singlet excited chlorophyll a, measured by fluorescence transients.

Keywords Circular dichroism spectroscopy · Diatoms · Electrochromic absorbance changes · Macrodomains · Light-harvesting complexes · *Phaeodactylum tricornutum* · Thylakoid membranes

Abbreviations

CD	Circular dichroism
Chl *a*	Chlorophyll *a*
Chl *c*	Chlorophyll *c*
DDE	Diadinoxanthin-deepoxidase
Ddx	Diadinoxanthin
DM	*n*-Dodecyl-*β*-D-maltoside
Dtx	Diatoxanthin
FCP	Fucoxanthin-chlorophyll *a/c* binding protein
Fx	Fucoxanthin
LD	Linear dichroism
LHC	Light-harvesting complex
NPQ	Non-photochemical chlorophyll fluorescence quenching
PAM	Pulse amplitude modulated chlorophyll fluorometer
PAR	Photosynthetically active radiation
PSI	Photosystem I
PSII	Photosystem II
psi-type	Polymerization or salt-induced

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Introduction

Diatoms play major roles in the primary biomass production in the phytoplankton communities of the marine seas and freshwaters. In their natural habitat, they are exposed to randomly fluctuating light intensities and thus evolved efficient light harvesting and photoprotection mechanisms.

The thylakoid membranes of this species are loosely appressed and do not differentiate into grana and stroma membranes. Instead, they are organized into groups of three (Berkaloff et al. 1990; Gibbs 1962; Pyszniak and Gibbs 1992). Their main light-harvesting complexes are the fucoxanthin-chlorophyll *a/c* proteins (FCPs), which serve as antenna for both photosystems (Owens 1986b). In contrast to the LHCII of higher plants, which is concentrated in the granal regions of the thylakoid membranes, the FCP of diatoms is evenly distributed (Pyszniak and Gibbs 1992).

FCPs belong to the LHC-superfamily and possess many structural similarities to LHCII, the main Chl *a/b* complex of photosystem II (Durnford et al. 1996; Grossman 1990). From the sequence, three membrane-spanning helices were predicted, from which helices 1 and 3 show considerable homology to LHCII (Eppard and Rhiel 1998). In FCPs Chl *b* is replaced by Chl *c* and the main light-harvesting carotenoid is fucoxanthin (Fx), instead of lutein. In the complex, Chl *c* is located in close vicinity to Chl *a*, and two fucoxanthins are probably arranged in a similar way, as the luteins in LHCII. Little is known about the arrangement and binding sites of the two other Fx molecules, since helix 2 exhibits only little homology with the respective helix 2 of LHCII (Papagiannakis et al. 2005; Wilhelm et al. 2006).

In higher plants, LHCII and LHCII-PSII complexes are assembled into chirally organized macrodomains, which exhibit intense polymerization or salt-induced (psi-type) circular dichroism (CD) signals (Garab 1996) that readily undergo light-induced reversible reorganizations (Barzda et al. 1996; Cseh et al. 2000). Moreover, these structural changes do not depend directly on the photosynthetic electron and proton transport (Istokovics et al. 1997), but depend linearly on the light intensity above the saturation of photosynthesis and were found to be sensitive to changes in the ambient temperature (Cseh et al. 2005).

There are only a few experimental data concerning the supramolecular organization of the LHC complexes of Chl *a/c*-containing organisms. In *Mantoniella squamata*, much weaker anomalous CD signals are present in the intact cells compared to higher plant chloroplasts, and no anomalous CD could be identified in the isolated LHC and thylakoid membranes (Goss et al. 2000). Intact cells and isolated chloroplasts of *Pleurochloris meiringensis* possess anomalous CD signals that are diminished when the chloroplasts are disrupted (Büchel and Garab 1997). Recently, oligomeric FCP complexes resembling the native state of the diatom antenna system have been isolated from *P. tricornutum* by biochemical means (Lepetit et al. 2007). For another diatom, *Cyclotella meneghiniana* (Büchel 2003), the existence of trimeric and higher oligomeric complexes has also been shown. These results suggest that different algal groups, which possess Chl *a/c*-containing LHCs, exhibit chirally organized macrostructures. However, our knowledge concerning the nature of this type of macro-organization of the complexes and their structural flexibility in diatoms is far less advanced than in granal chloroplasts and LHCII of higher plants.

In the present work, using mainly CD spectroscopy, we conducted a systematic study on the (macro-)organization of the pigment–protein complexes in *P. tricornutum* at different levels of complexity. The structural flexibility of the chiral macrodomains was tested by suspending the cells in different media with different osmolarities and ionic strengths, and by measuring their thermal and light stabilities and the ability of the chiral macrodomains to undergo light-induced reversible reorganizations. We also established correlations of these reorganizations with functional parameters, the maximum quantum yield, measured as F_v/F_m, and the non-photochemical quenching (NPQ) of the Chl *a* fluorescence.

Materials and methods

Phaeodactylum tricornutum cells (1090-1a), obtained from the Culture Collection of Algae, Göttingen (SAG, FRG), were grown in ASP-Medium according to Provasoli et al. (1957) with the modifications by Lohr and Wilhelm (2001). Cells were cultivated as batch cultures at a photon flux density of 40 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR) in a dark/light regime of 8/16 h. The temperature of the growth chamber was set to 19°C.

Isolation of thylakoid membranes was performed according to Büchel (2003) with minor modifications. Cells from the exponential growth phase with the chlorophyll concentration of 4–5 μg/ml were harvested by centrifugation (4,000*g*, 5 min). The following steps were carried out at 4°C in dim light. The pelleted cells were resuspended in isolation medium 'A' (10 mM MES pH = 6.5, 2 mM KCl, 5 mM EDTA, 1 M sorbitol) and disrupted in a French pressure cell (Thermo Scientific) at 12,500 psi (8.62×10^7 Pa). Unbroken cells were pelleted by centrifugation (1,000*g*, 10 min) and resuspended in isolation medium 'A' again. The suspension was subjected to the French-press for a second time using the same conditions. After centrifugation (1,000*g*, 10 min) the supernatant was merged with the supernatant of the first centrifugation and centrifuged at 40,000*g* for 20 min. The pelleted thylakoids

were resuspended in isolation medium 'B' (10 mM MES pH = 6.5, 2 mM KCl, 5 mM EDTA).

For solubilisation of the membranes, equal amounts of the isolated thylakoids, corresponding to 1 mg Chl, were centrifuged at 40,000*g* for 20 min. The pelleted thylakoid membranes were solubilized in *n*-dodecyl *β*-D-maltoside (DM; DM/chl = 20:1, Sigma) on ice for 20 min. The solubilized membranes were centrifuged at 40,000*g* for 20 min and the supernatant was loaded onto a continuous sucrose gradient (0–0.7 M sucrose, supplemented with 0.03% DM). Separation was carried out by ultracentrifugation (Sorvall, UltraPro 80) using a swing-out rotor (Sorvall, TH-641) at 110,000*g* for 18 h. Chl content was determined according to Jeffrey and Humprey (1975). The brown colored bands containing the FCP were harvested from the gradient and used for CD spectroscopy.

The CD spectra were measured (if not indicated otherwise) at room temperature in a Jobin-Yvon CD6 dichrograph in the wavelength range of 400–750 nm with a bandwidth of 2 nm. The Chl concentration was set to 20 μg/ml in the case of thylakoid membranes and FCP preparations and 15 μg/ml in the case of intact cells. The optical pathlength was 1 cm. The CD-spectra are plotted in absorbance units.

The measurements of temperature-dependent CD-changes of intact cells were performed in a thermostated sample holder of the dichrograph. The samples were preincubated at different temperatures in the range of 20–50°C in 5°C steps for 10 min and measured at the same temperature.

Kinetics of the light-induced CD-changes in the cells was measured at 698 nm in the presence of 7% ficoll to avoid sedimentation during the measurement. Illumination was performed with a side-illumination attachment (Barzda et al. 1996). The photomultiplier was protected with crossing filters against actinic light from the exciting beam. CD-changes were induced by blue light of 800 μmol m^{-2} s^{-1} photon flux density.

Sonication of intact cells was performed in a Branson Sonifier 450 on ice with a 10 s sonication – 30 s cooling cycle for different time periods as indicated in the 'Results and discussion' section. The suspensions of sonicated cells with a Chl concentration of 15 μg/ml were used for CD spectroscopy.

Absorbance spectra were recorded with a Zeiss Specord M500 spectrophotometer in the wavelength range from 400 to 750 nm. The bandwidth was set to 1 nm. The Chl concentration of the samples was adjusted to the same values as for CD measurements.

Electrochromic absorbance changes induced by single turnover flashes were measured at different wavelengths between 470 and 570 nm in a set-up described earlier (Büchel and Garab 1995). The time constant was set to 100 μs; 32 kinetic traces were collected with a repetition rate of 1 s^{-1} and averaged.

Intact and sonicated cells and isolated thylakoid membranes were visualized by Zeiss 902 electron microscope. The samples were pelleted in Eppendorf tubes, the pellet was fixed with 2% glutaraldehyde, postfixed with 1% osmium-tetroxide, dehydrated with ethanol and embedded in Araldite. Ultrathin sections were cut out in ultramicrotome (Leica Ultracut UCT) and stained with 2% uranyl acetate and lead citrate.

Room temperature fluorescence measurements were performed using a PAM 101 Chl fluorometer (Walz, Effeltrich, Germany) equipped with a Clark-type oxygen electrode, as a sample holder. The algal cell cultures and isolated thylakoids were used at a Chl concentration of 15 and 20 μg/ml, respectively. The fluorescence parameters F_0 and F_m were recorded after 45 min low light adaptation (20 μmol m^{-2} s^{-1}). (F_0 and F_m are the minimum and maximum fluorescence yield, respectively, in low light-adapted state.) The light intensity of saturating flashes was 4,000 μmol m^{-2} s^{-1}. The maximum quantum yield of PSII photochemistry of thylakoids in reaction buffers in the absence or presence of sorbitol and $MgCl_2$ was calculated as F_v/F_m, where $F_v = F_m - F_0$, called variable fluorescence. F_0 and F_m were determined after a 5 min incubation in the reaction medium, 50 mM HEPES-KOH pH = 7.5, which was complemented with either or both $MgCl_2$ (1–25 mM) and sorbitol (350 mM). During the NPQ measurements saturating light flashes (4,000 μmol m^{-2} s^{-1}) with duration of 700 ms were applied with 1 min intervals. The actinic light intensity was adjusted to 700 μmol m^{-2} s^{-1}. In the case of isolated thylakoid membranes, 40 mM ascorbate was added as a co-substrate for DDE to enable conversion of Ddx to Dtx and 200 μM methyl viologen was added as electron acceptor. NPQ was calculated after 10 min actinic light illumination using the Stern-Volmer data treatment ($NPQ = F_m/F_m' - 1$) according to Bilger and Björkman (1990). (F_m' is the maximum fluorescence yield in actinic-light adapted state.)

Results and discussion

In order to investigate the macro-organization of diatom FCP complexes at different organizational levels, intact cells, isolated thylakoid membranes and purified FCP, we used CD spectroscopy; for comparison, absorbance spectra were also recorded on the same samples (Fig. 1). In thylakoid membranes and FCPs the characteristic CD bands could be found at (+)445 nm, (–)478 nm and (–)679 nm. The band at 679 nm evidently originated from Chl *a* molecules, while the band pair at 445/478 nm is most likely given rise by excitonic interactions involving Chl *a* and Chl *c* or carotenoids. This assumption is also in line with absorption and fluorescence excitation measurements,

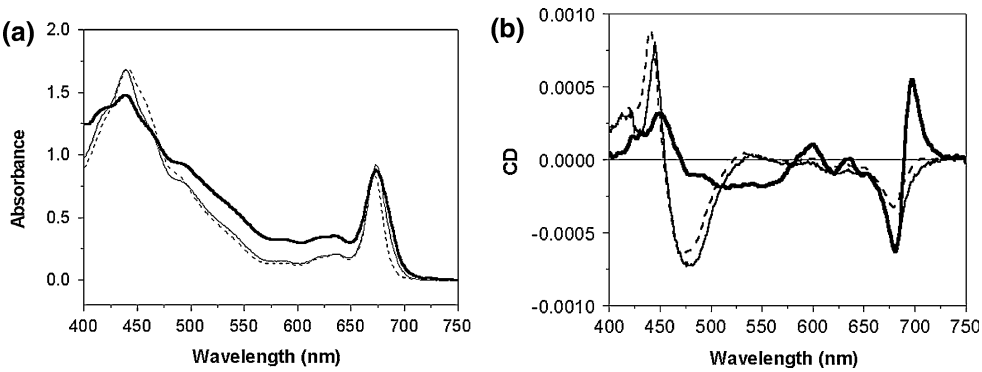

Fig. 1 Absorbance (**a**) and circular dichroism (CD) (**b**) spectra of intact cells (bold line), isolated thylakoid membranes (solid line) and isolated FCPs (dashed line) of *Phaeodactylum tricornutum*. The spectra are plotted for identical chlorophyll concentration of the samples (20 µg/ml)

which were reported for *P. tricornutum* (Owens 1986a) and the brown alga *Dictyota dichotoma* (Mimuro et al. 1990). The CD band at (–)478 nm was broad and probably contained contributions from Fx, the main light-harvesting pigment, as it was reported for brown algae (Katoh 1992). The nature of the (–)679 nm band is unclear. In *P. meiringensis* a similar band was found at (–)679 nm, also accompanied with a psi-type band at (+)693 nm. The very large (–)679 nm and (+)693 nm bands in whole cells and isolated chloroplasts of *P. meiringensis*, which were sensitive to the light intensity during growth, could be assigned to psi-type origin, while the much weaker but still intense (–)679 nm band in the Chl *a/c* LHC originated from a small fraction of Chl *a* which showed a bathochromic shift and a strong chirality, i.e., could be attributed to an induced intrinsic band of high intensity (Büchel and Garab 1997). In isolated FCP of *P. tricornutum* the (–)679 nm band was considerably smaller than in the thylakoid membranes and also the (+)445 nm band was shifted by 3 nm to shorter wavelengths, which probably indicates a more intact state of FCP complexes, or the presence of large, ordered aggregates in the native thylakoid membranes. In line with this notion, the intensity difference between the thylakoids and FCP, and in particular the decrease of the (–)679 nm band in isolated FCP could not be accounted for by CD contributions of the reaction center complexes, which exhibited characteristic bands at (+)506 nm and at (+)676 nm and (–)690 nm (data not shown). Surprisingly, in intact cells the excitonic bands at (+)445 nm and (–)478 nm were much weaker than in isolated FCPs and thylakoids. This might be caused by scattering artefacts in intact cells, the presence of which is evident in the absorbance spectra (Fig. 1a), or by an overlapping strong and broad psi-type band in the same region. Indeed, intact cells exhibited an intense band at (+)698 nm with psi-type features. Upon isolation of thylakoid membranes, this band disappeared together with the presumed broad band in the Soret region. The (+)698 nm psi-type band could be gradually diminished by sonicating the cells for different time periods. Sonication led to a gradual disorganization of the multilamellar membrane system. It also led to the apparent intensification of the (+)445/(–)478 nm band pair (Fig. 2). Similar increase of the excitonic bands representing Chl–Chl interactions has been reported in other Chl *a/c*-containing algae upon disruption of intact cells or isolated chloroplasts (Büchel and Garab 1997; Goss et al. 2000). The sensitivity of (+)698 nm band, and possibly also the disappearance of the putative broad band in the blue, further corroborated our conclusion on the origin of this (these) band(s) in chirally organized macrodomains. These macrodomains, in accordance with the theory of psi-type CD for large ordered three dimensional arrays (Garab 1996; Keller and Bustamante 1986), appeared to be associated with a multilamellar thylakoid membrane system in the cell. Upon isolation of thylakoid membranes, the loose stacking of membranes was lost, also sonication led to gradual disorganization of the membrane structure, as it was visible by electron microscopy (data not shown). It must be pointed out, however, that parallel running membrane sheets themselves, i.e., without a chiral organization of the complexes, cannot give rise to psi-type CD, as e.g. in bundle sheath chloroplasts (Faludi-Dániel et al. 1973).

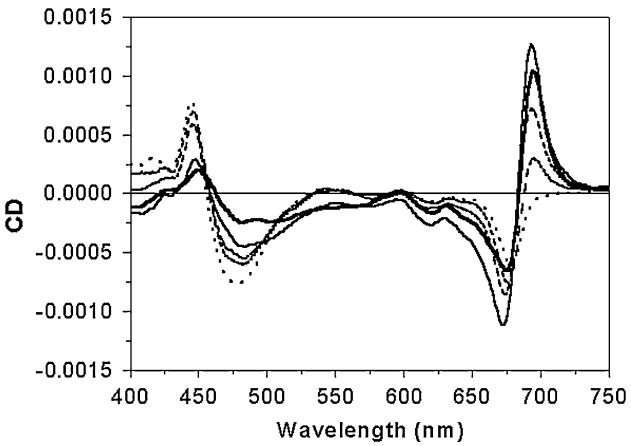

Fig. 2 Effect of sonication on the circular dichroism (CD) spectra of *Phaeodactylum tricornutum* cells. The same cell suspension was sonicated for 10 s (solid line), 60 s (dashed line), 90 s (dashed-dotted line) and 180 s (dotted line). The non-sonicated control is represented by bold line. Chlorophyll concentration, 15 µg/ml; optical pathlength, 1 cm

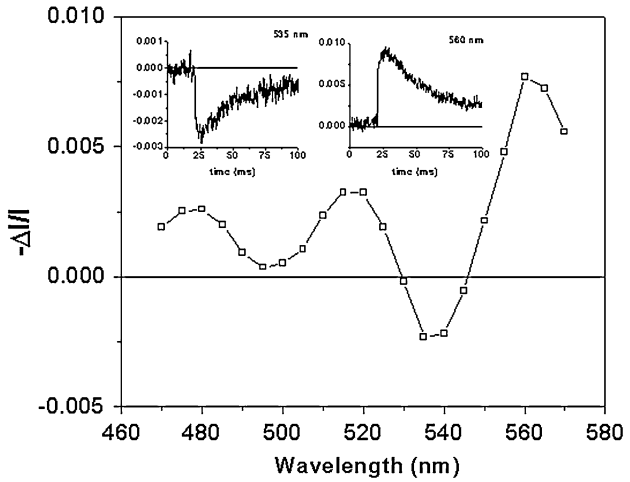

Fig. 3 Transient spectra of flash-induced absorbance changes, dominated by electrochromic changes, in dark-adapted intact cells of *Phaeodactylum tricornutum* 1 ms after the exciting flash. Insets: flash-induced absorbance transients at the indicated wavelengths

In order to gain further information about the orientation and local environment of pigment molecules absorbing in the green 'window,' we also analyzed the flash-induced electrochromic absorbance changes of intact cells of *P. tricornutum* between 470 and 570 nm. The transient absorbance spectrum depicted in Fig. 3 revealed a major positive band at 560 nm. This band, with its unusually long wavelength position, can evidently be assigned to a small fraction of Fx molecules with absorption maximum at around 545 nm. (The zero crossing in the transient absorbance spectrum corresponds to the absorption maximum of the electrochromic pigment.) The strong electrochromic response of these Fx molecules shows that they probably interact with Chl molecules, thereby lending a dipole moment to this molecule. It is also interesting to note that in higher plants the strongest electrochromic response, at around 515 nm, is attributed to lutein/Chl *b* interactions (Sewe and Reich 1977), suggesting that the long-wavelength absorbing, field-indicating lutein/Chl *b* pigment pair in the LHCII might be replaced by a similar Fx/Chl *c* pair in the FCP. (The strong absorption transient of the Fx molecules also indicated that these molecules were oriented in such a way in the thylakoid membrane that they were able to sense the electric field generated by the photosynthetic electron transport.) These data show that, similarly to higher plants, purple bacteria and Chl *a*/*c*-containing algae (Büchel and Garab 1995; de Grooth et al. 1980; Joliot and Joliot 1989; Kakitani et al. 1982), the electrochromic absorbance changes in *P. tricornutum* originate from a minor fraction of pigment molecules. These molecules with red-shifted but weak absorbance band, around 545 nm, exhibit virtually no CD signal but are most likely identical with those that exhibit a well discernible LD band (Hiller and Breton 1992). Further details of the molecular organization of this long-wavelength absorbing Fx might be possible to gain from Stark-spectroscopy (Palacios et al. 2004). It is interesting to note that a similar, weak, CD-silent long-wavelength (~535 nm) absorbing band was identified in a marine green alga, and was assigned to originate from a new electronic excited state, S_x between S_1 and S_2, of siphonaxanthin, which appears to transfer to a specific Chl *a* molecule(s) (Akimoto et al. 2004, 2007). This state arises only in pigment–protein complexes, probably due to a specific interaction with amino acids (Akimoto et al. 2007), and resembles the long wavelength absorbance band of Fx in diatoms (Gillbro et al. 1993).

In order to obtain information about the structural flexibility of the macrodomains, we subjected intact cells to different environmental conditions. Upon high light illumination, we observed a pronounced increase in the (+)698 nm band, which was accompanied by a smaller increase of the (–)679 nm CD-signal (Fig. 4a), while the

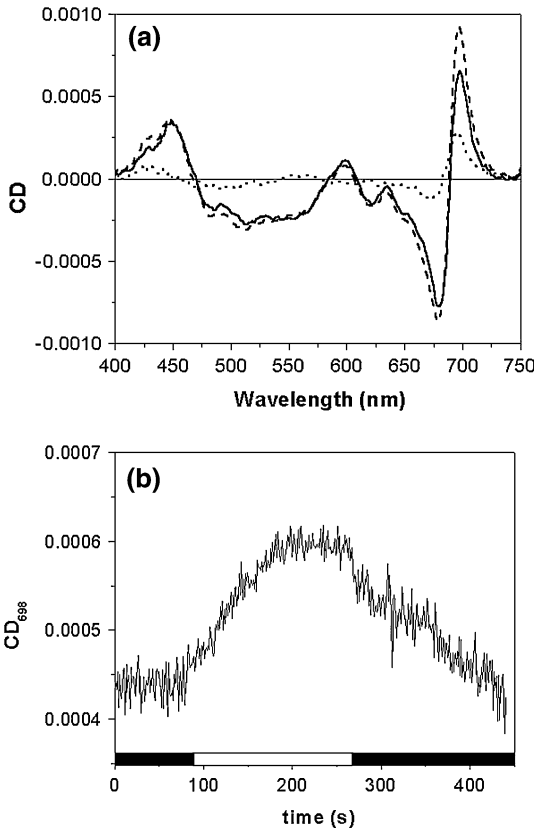

Fig. 4 Circular dichroism (CD) spectra of dark-adapted (solid line) and preilluminated (dashed line) *Phaeodactylum tricornutum* cells (**a**); dotted line, illuminated-minus-dark-adapted difference spectra. Time course of the light-induced CD-changes at 698 nm (**b**). Closed bars, dark periods; open bar, illumination period. The measurements were performed at room temperature; the light intensity of illumination was 800 μmol m^{-2} s^{-1}; chlorophyll concentration, 15 μg/ml; optical pathlength, 1 cm

other parts of the spectrum remained essentially unchanged. Kinetics of the CD-changes at 698 nm induced by high light illumination is shown in Fig. 4b. Illumination caused an increase in the CD-signal, which levelled off after around 100 s. After switching off the actinic light, the signal decreased to its original value, however, this phase was considerably slower (about 200 s) than the induction phase. In the blue region of the spectrum, no light-induced CD-changes could be observed (data not shown). This is in contrast to the thylakoid membranes of higher plants, where a characteristic CD band with psi-type features was found at 510 nm, which exhibited the same dependence on illumination or changes of the ambient temperature as the (+)688 nm band (Barzda et al. 1996). We also noticed that the amplitude of the (+)698 nm and (–)679 nm bands were influenced by the light intensity during growth of the alga culture (data not shown). Again, this behavior is similar to the behavior of the (+)693 nm and (–)679 nm bands in *P. meiringensis* (Büchel and Garab 1997).

In agreement with the observations in higher plants, the (+)698 nm psi-type band of *P. tricornutum* was also found to be sensitive to heat. By increasing the temperature up to 45°C, the (+)698 nm band decreased by 60%, while the main excitonic band pair remained essentially unchanged (Fig. 5). Between 45 and 50°C, there was a sharp decline in both amplitudes, indicating that in this temperature range the excitonic couplings between the FCP-bound pigments were also lost (Fig. 5). The increased sensitivity of the 698 nm band to heat indicates that the long-range chiral order of the complexes (chromophores) in intact cells possesses structural flexibility. These data also suggest that the macro-organization is prone to thermo-optically inducible reorganizations. However, presently we have no evidence for such an origin of the reorganizations in intact cells of *P. tricornutum.*

In granal thylakoid membranes two external factors influence the long-range chiral order of the chromophores: (i) electrostatic screening of the divalent cations, which facilitates the stacking of membranes, (ii) the osmotic pressure of the medium which influences mainly the lateral packing density of the complexes (Barzda et al. 1994; Garab et al. 1991). The long-range chiral order was found to be extremely sensitive to the osmolarity in different Chl *a/c*-containing organisms; in contrast, Mg^{2+} had no effect (Büchel et al. 1992; Büchel and Garab 1997; Goss et al. 2000). As shown in Fig. 6, upon increasing the sorbitol concentration in the medium, a large decrease in the long-range signal occurred. When the cells were resuspended in the normal culture medium again, the psi-type CD at 698 nm could be restored by about 80% of the original value (Fig. 6). It was interesting to observe that the reversible modulation of the CD signals was not confined to the psi-type CD, but also affected, albeit to somewhat lesser extent, the excitonic interactions in the Soret (data not shown). It was also interesting to note that an increase in the sorbitol concentration resulted in a red-shift of (–)679 nm band, which was also reversed after resuspending the cells in the culture medium. These results most probably indicate that variations in the osmotic pressure affect the lumenal space and thus the repeat distances in the multilamellar membrane system. This can lead to alterations in the

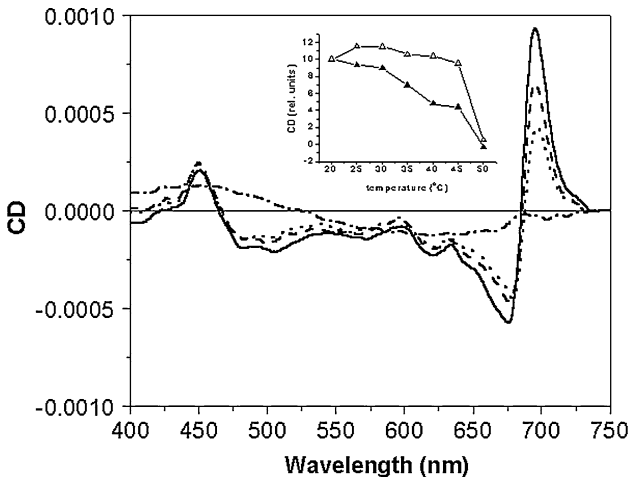

Fig. 5 Effect of the incubation temperature on the circular dichroism (CD) spectra of intact *Phaeodactylum tricornutum* cells. Cells were incubated consecutively for 10 min at 20°C (solid line), 35°C (dashed line), 40°C (dotted line) and 50°C (dashed-dotted line) and measured at the same temperature. Inset: Temperature dependence of the circular dichroism (CD) spectra at 698–750 nm (closed triangles) and at 450–470 nm (open triangles). Chlorophyll concentration, 15 μg/ml; optical pathlength, 1 cm

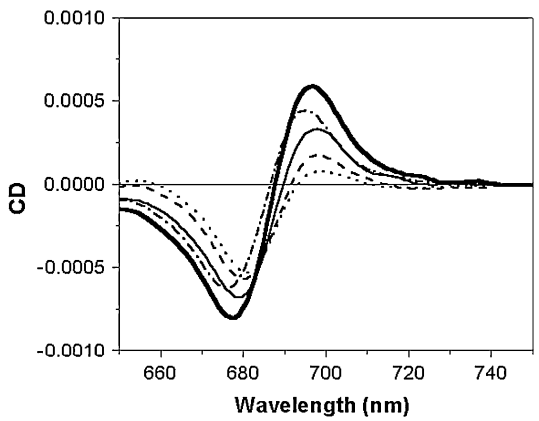

Fig. 6 Dependence of the circular dichroism (CD) spectra of intact *Phaeodactylum tricornutum* cells on the osmolarity of the medium. The cells were suspended in the culture medium (bold line) and in the same medium supplemented with 200 mM (solid line), 400 mM (dashed line) and 600 mM sorbitol (dotted line). The spectrum plotted with dashed-dotted line was recorded on cells suspended in the culture medium following their incubation in the 600 mM sorbitol-containing medium. Chlorophyll concentration, 15 μg/ml; optical pathlength, 1 cm

supramolecular array of the complexes, during which, however, excitonic interactions might also be affected.

Previously it was found that divalent cations (i.e., Mg^{2+}) are not able to influence the CD bands in *P. meiringensis*; this was attributed to the lack of stacked thylakoid membranes in these algae (Büchel and Garab 1997). In agreement with these observations, we also found that Mg^{2+} had no effect on the CD spectrum, as well as on the quantum yield of Chl fluorescence and the extent and kinetics of NPQ of intact *P. tricornutum* cells (data not shown). The effect of Mg^{2+} on intact cells, however, might have been hampered by the impermeability of the cell wall and the plastid envelope to the Mg-ions. Indeed, the situation was different in isolated thylakoid membranes. In these experiments we used thylakoid membranes that were suspended in HEPES buffer as a control. As shown in Fig. 7a, in the presence of Mg^{2+}, both in the presence or absence of sorbitol, characteristic changes could be observed: the negative CD in the region between 500 and 550 nm became more intense, along with the (–)679 nm band. In the region around 698 nm a small peak appeared

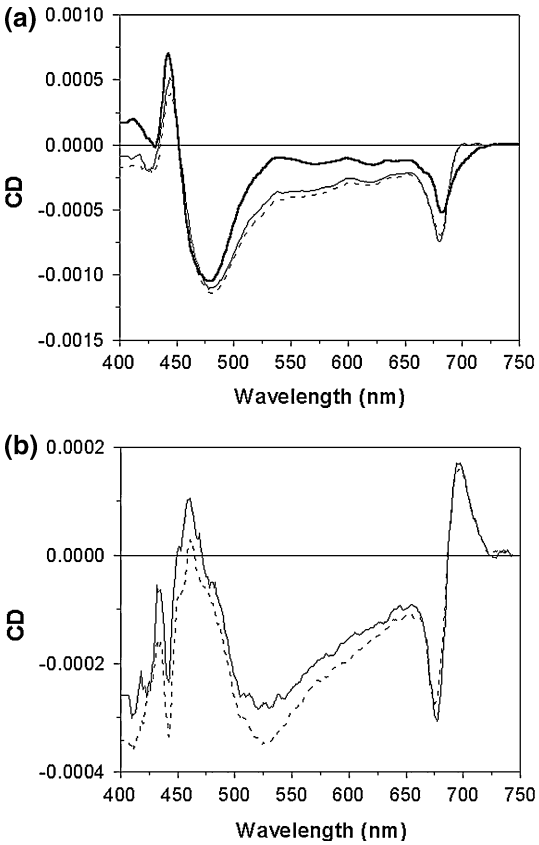

Fig. 7 Circular dichroism (CD) spectra of isolated thylakoids suspended in different media (**a**), and the corresponding difference spectra (**b**). Thylakoids were suspended for 5 min in 50 mM HEPES (bold line), 50 mM HEPES + 25 mM $MgCl_2$ (solid line) and 50 mM HEPES + 25 mM $MgCl_2$ + 350 mM sorbitol (dashed line). Chlorophyll concentration, 20 μg/ml; optical pathlength, 1 cm

which was completely absent in thylakoids incubated in the HEPES buffer. The difference spectrum clearly shows the changes introduced by the addition of Mg-ions (Fig. 7b); it remarkably resembles the CD spectrum of intact cells. This indicates that Mg^{2+} was able to partially restore the native structure of the thylakoid membrane, i.e., the macrodomain organization of the complexes.

In higher plants, divalent cations play an important role in membrane stacking, thus preventing the 'spillover' of excitation energy from PSII to PSI (for a recent review see Chow et al. 2005). The separation of PSII and PSI leads to an increase in the fluorescence yield of chloroplasts (Telfer et al. 1976). Mg-ions have also been shown to increase the maximum quantum yield of PSII and its capacity for NPQ, the non-photochemical fluorescence quenching (Noctor et al. 1993; Rees and Horton 1990). As shown in Table 1, Chl fluorescence parameters of the thylakoid membranes of *P. tricornutum* were also sensitive to Mg^{2+}. In general, the F_v/F_m values were considerably lower in isolated thylakoid membranes than in intact cells, indicating structural deteriorations during isolation, observations consistent with the CD data. Addition of Mg^{2+} caused an increase in F_v/F_m by about 35%, while F_0 decreased by about 12%, indicating a partially restored association of FCP with the reaction center complexes. The additional presence of sorbitol led to a further increase of the maximum quantum yield, but the effect of sorbitol was not as pronounced as that of Mg^{2+}.

We have also tested the effect of $MgCl_2$ on the NPQ. The overall NPQ in isolated thylakoids was always considerably lower than in intact cells, again evidently due to impairments caused by the isolation procedure (Table 1). Isolated thylakoid membranes of diatoms readily lose their whole chain electron transport activity, no matter how

Table 1 Chlorophyll fluorescence parameters of intact cells and isolated thylakoid membranes of *Phaeodactylum tricornutum* suspended in different media[a]

	F_0	F_m	F_v/F_m	NPQ
HEPES	0.20	0.23	0.17	0.03
HEPES + $MgCl_2$	0.17	0.27	0.37	0.19
HEPES + Sorbitol	0.20	0.27	0.29	0.10
HEPES + $MgCl_2$ + Sorbitol	0.17	0.28	0.39	0.26
Intact cells	0.12	0.38	0.69	1.13

[a] The membranes with a chlorophyll concentration of 20 μg/ml were suspended for 5 min in 50 mM HEPES-containing reaction buffers supplemented with 25 mM $MgCl_2$ and 350 mM sorbitol. The light intensity of saturating pulse and actinic light illumination was 4,000 and 700 μmol m^{-2} s^{-1}, respectively. F_0 and F_m are the minimum and maximum fluorescence yield, respectively, in low light-adapted state. The maximum quantum yield of PSII photochemistry was calculated as F_v/F_m, where $F_v = F_m - F_0$, called variable fluorescence. The non-photochemical quenching (NPQ) values were determined after 10 min actinic light illumination as $F_m/F_m' - 1$. F_m' is the maximum fluorescence yield in actinic-light adapted state. $n = 5$, SD < 10%

carefully they are prepared and what isolation method is used (Martinson et al. 1998; Jakob and Goss unpublished). Furthermore, isolated thylakoid membranes are unable to accumulate large proton gradients—the membranes are leaky, as indicated by the absence of electrochromic absorbance changes in the ms time range. As a consequence, we were also not able to accumulate substantial amounts of Dtx (data not shown). Nevertheless, the magnitude of NPQ increased significantly in thylakoids in the presence of Mg^{2+}. Again, sorbitol had little or no effect. Hence, it can be concluded that Mg-ions not only restore, at least partly, the macrostructure of the thylakoid membranes, but also enhance the light-harvesting and quenching capacity of the membranes. Interestingly, while Mg^{2+} plays the main role in restoring the macrodomain organization, sorbitol also exerts positive effects on the Chl fluorescence parameters related to PSII photochemistry and the NPQ (Table 1). This indicates, that both sorbitol and Mg^{2+} play roles in the preservation of the structure and function of thylakoid membranes.

Conclusion

Our data show that the pigment–protein complexes in *P. tricornutum* are organized in large chiral domains, which give rise to psi-type CD signals. This macro-organization was sensitive to the temperature, the osmotic pressure and could be modulated by illumination of whole cells or by varying the light intensity during growth. The role of Mg^{2+} in the macro-organization of the complexes could be demonstrated in isolated thylakoid membranes. Correlations were revealed between the Mg^{2+}-assisted macro-organization and some functional parameters, F_v/F_m and NPQ. As concerns the molecular organization of FCP, it has been shown to contain an induced intrinsic CD in the red, giving rise to Chl *a* molecules with acquired chirality. As revealed by electrochromism measurements, a small fraction of Fx molecules exhibit a long-wavelength absorbance and extreme sensitivity to the transmembrane electric field, most likely due to local changes by a nearby Chl molecule.

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