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Biochimica et Biophysica Acta 1457 (2000) 190–199

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

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Organization of the pigment molecules in the chlorophyll *a*/*b*/*c* containing alga *Mantoniella squamata* (Prasinophyceae) studied by means of absorption, circular and linear dichroism spectroscopy

Reimund Goss [a,*], Christian Wilhelm [a], Győző Garab [b]

[a] *Institut für Botanik, Universität Leipzig, Johannisallee 21–23, 04103 Leipzig, Germany*
[b] *Institute of Plant Biology, Biological Research Centre, Hungarian Academy of Sciences, P.O. Box 521, H-6701 Szeged, Hungary*

Received 29 June 1999; received in revised form 12 January 2000; accepted 11 February 2000

Abstract

In order to obtain information on the organization of the pigment molecules in chlorophyll (Chl) *a*/*b*/*c*-containing organisms, we have carried out circular dichroism (CD), linear dichroism (LD) and absorption spectroscopic measurements on intact cells, isolated thylakoids and purified light-harvesting complexes (LHCs) of the prasinophycean alga *Mantoniella squamata*. The CD spectra of the intact cells and isolated thylakoids were predominated by the excitonic bands of the Chl *a*/*b*/*c* LHC. However, some anomalous bands indicated the existence of chiral macrodomains, which could be correlated with the multilayered membrane system in the intact cells. In the red, the thylakoid membranes and the LHC exhibited a well-discernible CD band originating from Chl *c*, but otherwise the CD spectra were similar to that of non-aggregated LHC II, the main Chl *a*/*b* LHC in higher plants. In the Soret region, however, an unusually intense (+) 441 nm band was observed, which was accompanied by negative bands between 465 and 510 nm. It is proposed that these bands originate from intense excitonic interactions between Chl *a* and carotenoid molecules. LD measurements revealed that the Q_Y dipoles of Chl *a* in *Mantoniella* thylakoids are preferentially oriented in the plane of the membrane, with orientation angles tilting out more at shorter than at longer wavelengths (9° at 677 nm, 20° at 670 nm and 26° at 662 nm); the Q_Y dipole of Chl *c* was found to be oriented at 29° with respect to the membrane plane. These data and the LD spectrum of the LHC, apart from the presence of Chl *c*, suggest an orientation pattern of dipoles similar to those of higher plant thylakoids and LHC II. However, the tendency of the Q_Y dipoles of Chl *b* to lie preferentially in the plane of the membrane (23° at 653 nm and 30° at 646 nm) is markedly different from the orientation pattern in higher plant membranes and LHC II. Hence, our CD and LD data show that the molecular organization of the Chl *a*/*b*/*c* LHC, despite evident similarities, differs significantly from that of LHC II.

Keywords: Alga; Carotenoid; Light-harvesting complex; Linear dichroism; Circular dichroism; Photosynthesis

1. Introduction

The photosynthetic apparatus of the prasinophycean alga *Mantoniella squamata* exhibits some unique properties that clearly distinguish it from those of higher plants and chlorophyll (Chl) *a*/*c*-containing algae. In *Mantoniella* chloroplasts, no differentiation

Abbreviations: CD, circular dichroism; Chl, chlorophyll; DM, dodecyl maltoside; LD, linear dichroism; LHC, light-harvesting complex; LHC II, the main higher plant Chl *a*/*b* LHC; PS, photosystem

* Corresponding author. Fax: +49-341-97-36899; E-mail: rgoss@rz.uni-leipzig.de

0005-2728/00/$ – see front matter
PII: S0005-2728(00)00101-8

has been observed between grana and stroma thylakoids, the thylakoids appear to be multilayered, with a parallel alignment of the membranes, separated by a gap of 2–8 nm [1]. *Mantoniella* contains only one functional light-harvesting complex (LHC), which serves as an antenna for both photosystems (PSs) [2]. Minor Chl *a*/*b*-binding proteins that are typical of the light-harvesting system of higher plants and green algae (for a recent review concerning pigment–protein complexes of PS I and PS II, see Green and Durnford [3]) are not present in *M. squamata*, although a multigene family of LHC genes has been found [4]. The LHC of *Mantoniella* displays no immunological relationship to the light-harvesting proteins of Chl *a*/*c*-containing brown algae [5] and Chl *a*/*b*-binding green algae or higher plants [6]. On the basis of the amino acid sequence, Rhiel and Mörschel [4] calculated a molecular weight of 24.2 kDa for the mature LHC polypeptide, while Schmitt et al. [7] reported a value of 21.3 kDa. The LHC polypeptides of *Mantoniella* exhibit a higher homology to the Chl *a*/*b*-binding antenna proteins (34–38% homology) [8,9] than to the Chl *a*/*c* LHCs (only 22% homology) [10]. Homology to the LHC II of higher plants is high in the C-terminal region containing helix III, but in consequence of the existence of atypical hydrophilic domains, helix I is not detected by predictive methods [4].

The organization of the light-harvesting systems in Chl *a*/*c*-containing algae differs comparably markedly in the different algal groups. In most of the chromophytic algae which have been analyzed so far, only one membrane intrinsic LHC can be found [11], though Büchel and Wilhelm [12] have reported the existence of two different LHCs, (associated with the two PSs) in *Pleurochloris meiringensis*, a member of the Xanthophyceae. Knoetzel and Rensing [13] also found PS I and PS II specific intramembrane antenna proteins in the marine dinoflagellate *Gonyaulax polyedra*.

Although the Chl *a*/*c*-containing algae differ considerably as concerns the accessory pigments in the different algal classes, the number of pigment species bound per polypeptide is relatively low. In diatoms, 3–4 different xanthophylls, one carotene and Chl *c* are to be found (for a detailed analysis of pigments in algal photosynthesis, see Rowan [14]). Higher plants and green algae contain up to five different xanthophylls, one carotene and Chl *b* as accessory pigments.

With regard to pigment binding, the LHC of *M. squamata* differs appreciably as compared to both Chl *a*/*b* and Chl *a*/*c* complexes. *Mantoniella* LHC binds three Chl species (Chl *a*, Chl *b* and Chl *c*), and up to 12 carotenoids, with prasinoxanthin being the main carotenoid light-harvesting pigment [15,16].

Data on the organization of the pigment molecules in the photosynthetic apparatus of Chl *a*/*c*-containing algae are scarce, and to our knowledge are not available at all for Chl *a*/*b*/*c* complexes. No crystallographic structural data have been reported on Chl *c*-containing LHCs, and our knowledge is therefore at a far less advanced state than that for LHC II [17] or related complexes. Information concerning the molecular organization of the complexes is derived mainly from circular dichroism (CD) and linear dichroism (LD) measurements, which have contributed significantly to our knowledge of the molecular organization of virtually all main pigment–protein complexes of higher plants and green algae [18,19]. Hiller et al. [20] utilized LD spectroscopy to clarify the orientation of Chl *a*, Chl *c* and the xanthophylls in a cryptophyte alga, *Chroomonas* CS24. Hiller and Breton [21] also reported on the light-harvesting system of two fucoxanthin–Chl *a*/*c*-containing algae, *Pavlova lutherii* and *Phaeodactylum tricornutum*. The organization of photosynthetic pigments and pigment–protein complexes of another diatom, *Cylindrotheca fusiformis*, has been investigated by Hsu and Lee [22]. In recent studies involving the use of CD and LD techniques, Büchel and Garab [23,24] concluded that the pigment organization of the LHC of *P. meiringensis* (Xanthophyceae) differs significantly from that of LHC II and also from that of the LHC in some other Chl *a*/*c*-binding algae. In the present study, absorption, LD and CD spectroscopy were applied to obtain data on the pigment organization of the photosynthetic apparatus of *M. squamata*. We found that, although some similarities are evident, the molecular organization of the Chl *a*/*b*/*c* LHC differs significantly from that of LHC II in higher plants.

2. Materials and methods

2.1. Plant material and cultivation

M. squamata Manton et Parke (isolated by Desikachary, supplied by the Culture Collection Plymouth, Cambridge, UK, strain LB 1965/1) was grown in batch cultures in artificial seawater medium according to Müller [25]. The light intensity during cultivation was 20 μmol photons m^{-2} s^{-1} under a light:dark regime of 14:10 h. The temperature of the growth chamber was held constant at 20°C.

2.2. Isolation of thylakoid membranes

Mantoniella cells were harvested from the logarithmically growing culture at a Chl content of 2 mg l^{-1} by centrifugation (3000×*g* for 5 min). Cells were resuspended in an isolation buffer containing 40 mM HEPES (pH 7.4, KOH), 10 mM KCl, 2 mM $MgCl_2$, 1 mM EDTA and 0.35 M sorbitol and immediately broken (1 min, 0°C) in a glass bed homogenizer (MSK, Braun, Germany). The homogenate was passed through a glass filter (pore size D1, Schott, Germany) and centrifuged at 3000×*g* for 5 min at 4°C. The pellet enriched in chloroplasts was washed with isolation medium and, after a further centrifugation step, osmotically shocked by a 2 min incubation in shock medium containing 10 mM HEPES (pH 7.4, KOH), 10 mM KCl and 2 mM $MgCl_2$. The thylakoid membranes were centrifuged (3000×*g*, 5 min, 4°C) and the resulting pellet was resuspended in a small volume of shock medium and kept on ice until the spectroscopic measurements.

2.3. Isolation of LHC

Thylakoid membranes of *M. squamata* were solubilized for 30 min on ice with dodecyl maltoside (DM) at a ratio of 30 mg DM per mg Chl. Solubilized thylakoids were spun for 15 min at 40 000×*g* at 4°C to remove unsolubilized membrane fragments. The supernatant was then loaded onto a discontinuous sucrose gradient consisting of six layers of different sucrose concentrations (10–35% sucrose in shock medium pH 7.4, 0.05% DM). Ultracentrifugation was performed with a swing-out rotor (Beckman SW 28) for 16 h at 100 000×*g* at 4°C. After separation, the purified LHC was found in the 20–25% sucrose layer of the gradient; it was carefully removed and stored on ice until the measurements.

2.4. Chl determination

Chl contents were determined spectrophotometrically in 80% acetone, the equations of Ziegler and Egle [26] being used.

2.5. Spectroscopy

Absorption spectra were recorded with a Specord M 500 spectrophotometer (Zeiss, Germany) in the wavelength range between 400 and 750 nm, using a bandpass of 1 nm. LHC and isolated thylakoid membranes were adjusted to a Chl content of 10 mg l^{-1}. Intact cells of *Mantoniella* were taken directly from the growing cultures at a Chl content of 2 mg l^{-1}. This explains the poor signal to noise ratio in the CD spectra of the intact cells (centrifugation induced significant alterations in the CD spectra).

CD spectra from 400 to 750 nm were measured at room temperature with a Jobin–Yvon (Longjumeau, France) CD6 dichrograph, using a bandpass of 2 nm. The CD spectra of the samples were corrected for the baseline of the culture medium in the case of intact *Mantoniella*, and for the baseline of the shock medium in the case of isolated thylakoids and purified LHC. CD is expressed in units of absorbance.

LD measurements were performed at room temperature between 400 and 700 nm with a Jobin–Yvon CD6 dichrograph equipped with an additional modulator board optimized for LD measurements; the bandpass was set to 2 nm. The membranes and isolated LHC were aligned by using the gel squeezing technique described by Abdourakhmanov et al. [27]. Concentrated samples of isolated thylakoid membranes or purified LHC were diluted with stock solutions of shock medium pH 7.4, acrylamide and *N,N′*-methylenebisacrylamide to yield a final Chl concentration of 10 mg l^{-1}. The final concentrations of the reaction mixture were 10% (w/v) acrylamide, 0.33% (w/v) *N,N′*-methylenebisacrylamide, 0.1% (v/v) *N,N,N′,N′*-tetramethylethyldiamide and 0.1% (w/v) ammonium persulfate. The samples were oriented by uniaxially squeezing the gel blocks to 50% of their

original length. Comparison of the absorption and CD spectra of the samples in polyacrylamide gel with the respective spectra in the buffer demonstrated that the polyacrylamide gel did not noticeably affect the molecular organization of the pigment molecules.

The orientation parameter S and the mean orientation angles of the pigments in the thylakoid membranes and in the isolated LHC were calculated according to [18].

3. Results

Fig. 1 reveals that the absorption spectra of intact *M. squamata* cells and the isolated thylakoid membranes are dominated by the absorbance of the LHC, which contains about 70% of the total Chl *a* content of the membranes [28]. The principal absorption bands of Chl *a* and Chl *b* are found at 675 nm and 646 nm, and 437 nm and 470 nm, respectively. The spectral contributions of the carotenoids at 486 and 525 nm could be identified most clearly in the absorption spectrum of the LHC. The 525 nm band has been assigned to prasinoxanthin, the main light-harvesting pigment of *M. squamata* [16]. Chl *c*, which has absorption maxima at around 450 and 630 nm, is present in significant amounts in *M. squamata*, but its absorption bands were not resolved because of the overlap with the more intense Chl *a* and Chl *b* bands in the Soret and the red spectral regions, respectively.

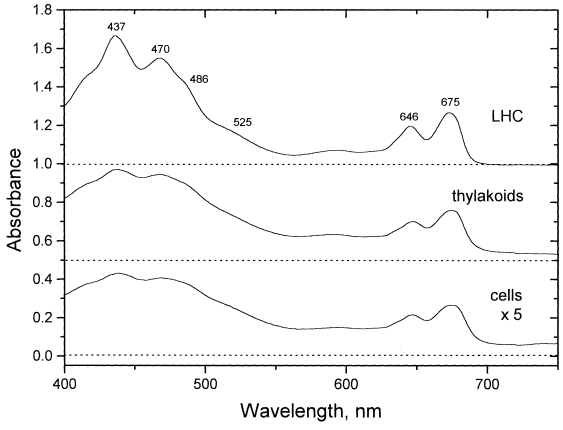

Fig. 1. Absorption spectra of intact cells, isolated thylakoids and purified LHC of *M. squamata*. The Chl concentrations were 2 μg ml^{-1} (cells) and 10 μg ml^{-1} (thylakoids, LHC). The absorption spectra of the thylakoids and LHC are shifted along the *y* axis; zero absorbances are indicated by broken lines.

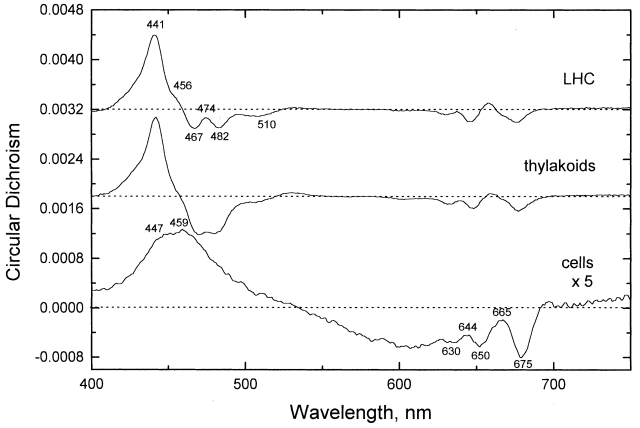

Fig. 2. CD spectra of intact cells, isolated thylakoids and purified LHC of *M. squamata* recorded at a Chl concentration of 10 μg ml^{-1} for the thylakoids and LHC and 2 μg ml^{-1} for the intact cells. The spectra of the thylakoids and LHC are shifted along the *y* axis, as indicated by the broken lines.

CD measurements (Fig. 2) on intact cells of *Mantoniella* in the Soret region revealed a shoulder at 447 nm and a broad positive band with a peak at 459 nm. An additional band could be seen at around 520 nm, but it was not resolved clearly because of the presence of the long tail above 500 nm. As evident from the anomalous shape of the main band and from a comparison with the CD spectra of the thylakoids and LHC, the bands cannot be assigned to excitonic CD, but are likely to arise from some kind of chiral macroarray [18]. In the red, three negative bands at 630, 650 and 675 nm and two positive bands at 644 and 665 nm were identified in the case of the intact cells. On the basis of the absorption spectra and pigment stoichiometries of *Mantoniella* cells, the (−) 630 nm and the (−) 675 nm peaks could be assigned to Chl *c* and Chl *a*, respectively.

Upon isolation of the thylakoids and LHC, the anomalously shaped band peaking at 459 nm essentially disappears and the (−) 675 nm band becomes considerably weaker. These changes are probably correlated with the loss of the regular, multilayered, membrane system.

It can be seen that the CD spectra of the thylakoids and the LHC closely resemble each other, evidently because of the substantial contribution of the LHC to the total pigment content of the membranes. In the red, the band structure of the thylakoids and LHC of *Mantoniella* also resembles the band composition for higher plant thylakoids and non-aggre-

gated LHC II. This similarity is most evident between 650 and 700 nm, where the composition and magnitude of the CD bands ((−) 650, (+) 665 and (−) 675 nm) are similar to those in LHC II [29,30]. This suggests a similar molecular organization in the Chl *a*/*b*/*c* LHC and in Chl *a*/*b* LHC II, at least as far as the interactions between the Q_Y dipoles of the Chl *a* and *b* molecules.

In the Soret region, some similarities remain between the LHC and LHC II. The bands at (−) 467 and (+) 474 nm may originate from excitonic interactions involving Chl *b* molecules, similarly as in LHC II. The (−) 482 nm band can be assigned to a carotenoid, the peak position of which, however, is clearly different from that of the corresponding band in LHC II (cf. [31]). The origin of the shoulder at 456 nm is unclear, though it may arise from Chl *c* molecules. The band above 500 nm originated from carotenoids, and most likely from prasinoxanthin, exhibits a pronounced shoulder in the absorption spectrum in this wavelength region [16]. In the Soret region, however, the CD spectrum is characterized by dissimilarity rather than by similarity between the two types of complexes. The intensity of the positive CD band at 441 nm is about an order of magnitude higher than that of similar bands in LHC II or the thylakoid membranes (cf. e.g. [32,33]) and Chl *a*/*c* LHC [23]. This band evidently originates from Chl *a*; it is red-shifted by about 4 nm as compared to the absorption band. Similar, red-shifted, very intense, non-conservative CD has been shown to result from the Q_Y transition of a small number of Chl *a* molecules in *P. meiringensis* [23] and is attributed to an induction of chirality in Chl *a* by its binding to the apoprotein. In *Mantoniella*, a similar explanation can be ruled out, because induction of the chirality of the molecule should cause significant CD not only around 440 nm, but also at all other transitions. On the other hand, the (+) 441 nm CD band is accompanied by negative bands above 460 nm which evidently originate from carotenoids. The integrated areas of the positive and the negative bands appear to be approximately equal. This strongly suggests that the bands between 440 and 510 nm originate from excitonic interactions between Chl *a* and carotenoid molecules. Further experiments concerning the nature and possible role of these interactions are in progress.

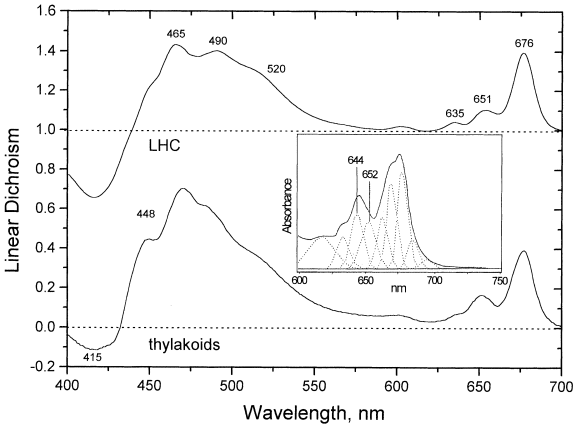

Fig. 3. LD spectra of isolated thylakoids and purified LHC of *Mantoniella* embedded in polyacrylamide gel and aligned by uniaxial squeezing; Chl concentration 10 μg ml^{-1}. The spectrum of the LHC is shifted along the *y* axis, zero dichroism signals are indicated by broken lines. Inset: absorption spectrum of intact cells recorded at 77 K at a Chl concentration of 2 μg ml^{-1}. The fitted Gaussian bands are given by dotted lines.

The isolated thylakoid membranes and purified LHC of *M. squamata* exhibited strong LD signals (Fig. 3), which clearly indicated that the pigments possess a non-random orientation with respect to the membrane plane and to the main axes of the isolated complexes. In the red, the thylakoid membranes displayed strong positive LD at 676 and 651 nm and a weaker maximum at 635 nm. These LD bands can be assigned to Chl *a*, Chl *b* and Chl *c* molecules, respectively, and demonstrate that the Q_Y dipoles of these molecules preferentially lie in the membrane plane. The orientation of the Chl *a* Q_Y pattern dipoles in Chl *a*/*b*/*c* LHC agrees well with the pattern found in higher plants (cf. [18,19]). The mean orientation angles of the Chl *a* transitions in the red part of the spectrum are 9° (677 nm), 20° (670 nm) and 26° (662 nm) with respect to the plane of the thylakoid membrane (Table 1). In contrast with the thylakoid membranes of higher plants, the strong positive LD band peaking at 651 nm indicates that the Q_Y transition dipoles of Chl *b* in *Mantoniella* preferentially possess an in the plane orientation, with a calculated orientation angle of 23° with respect to the plane of the membrane. In higher plants, these dipoles of Chl *b* have been shown to tilt out of the membrane plane [34,35]. Gaussian analysis of the low-temperature absorbance spectrum of intact *Mantoniella* cells (inset in Fig. 3) showed the presence of

Table 1
The orientation parameter *S* and the mean orientation angles of the transition dipoles of the photosynthetic pigments in the thylakoid membranes and isolated LHC of *M. squamata*

Pigment	Wavelength (nm)	Thylakoids		LHC	
		S (orientation parameter)	Mean orientation angle (°)	*S* (orientation parameter)	Mean orientation angle (°)
Chl *a*	677	0.20	9	0.15	19
	670	0.14	20	0.07	28
	662	0.09	26	0.03	32
Chl *b*	653	0.12	23	0.05	30
	646	0.09	26	0.02	33
Chl *c*	635	0.06	29	0.02	33
Carotenoids	520	0.19	11	0.10	25
	490	0.19	11	0.07	28

The orientation parameter *S* and the orientation angles were calculated according to [18]. The orientation angles of the pigments are given with respect to the plane of the thylakoid membrane or the axis of the isolated complex, respectively.

two Chl *b* forms absorbing at 652 nm and 644 nm. The Chl *b* molecules absorbing at 644 nm exhibit a slightly larger orientation angle (26°), but also contribute significantly to the positive LD in the Chl *b* region.

The positive LD around 635 nm can be assigned to Chl *c*, and indicates a preferentially in the plane orientation of the Q_Y dipoles of these molecules. The calculated orientation angle for the Q_Y transition of Chl *c* is 29° with respect to the plane of the thylakoid membrane. Different results have been reported regarding the orientation of Chl *c*. In the fucoxanthin–Chl *a*/*c* complex from the brown alga *Dictyota dichotoma*, no Chl *c* LD band could be identified [36], whereas Hiller et al. [20] found a form of Chl c_2 giving a strong positive LD band at 645 nm in the LHC of *Chroomonas* CS24.

The thylakoid membranes exhibited a negative LD band at 415 nm and positive contributions at 448, 465, 490 and 520 nm. The 415 nm LD band evidently originates from Chl *a*, as in LHC II. The shoulder at 448 nm and the positive LD band at 465 nm can most probably be assigned to Chl *c* and Chl *b*, respectively. Similarly to the Soret bands of Chl *a*, the Chl *b* and *c* transitions probably reflect simultaneous excitations of two or more absorption transition dipole moments [37], and the interpretation of these bands may therefore be complex. The bands at 520 and 490 nm can be assigned to long-wavelength-absorbing carotenoids such as prasinoxanthin and other carotenoids that absorb at shorter wavelengths, respectively. These carotenoid molecules are preferentially oriented in the plane of the thylakoid membrane, with orientation angles of 11° for both the long- and the short-wavelength-absorbing pigments. It is interesting that the relative LD amplitudes of the positive bands in the blue with respect to the corresponding LD signals in the red are considerably higher in *Mantoniella* than in higher plant thylakoids. It seems likely that this can be accounted for by a more significant contribution from the carotenoids in Chl *a*/*b*/*c* LHC than in LHC II.

The isolated LHC exhibited a more pronounced negative LD band at 415 nm than that for the thylakoids, and the zero crossing was shifted toward longer wavelengths. The explanation of these differences is uncertain. It could be due to the lack of a positive LD contribution at around 440 nm. A band with intense positive LD at 440 nm in *P. meiringensis* has been assigned to PS I [24]. In other regions, as demonstrated by the close similarity of the two spectra, the LD spectrum of the thylakoids was dominated by the dichroism of LHC. The fact that the LD of the purified LHC has the same sign as the LD of the isolated thylakoids means that the mechanism of alignment of the LHC in the gel is essentially the same as that for the thylakoids, suggesting that the isolated complexes form trimers or small disc-shaped aggregates. The trimerization of *Mantoniella* LHC was proposed by Rhiel et al. [38], who used electron microscopy to determine the native structure of the light-harvesting system. The values of the orientation

parameters for the LHC (Table 1) are somewhat lower than those for the thylakoids, which can most probably be attributed to irregularities in the shape of the LHC trimers or oligomers. The carotenoid molecules display a significantly lower degree of orientation with respect to the plane of the isolated complex than that in the thylakoids (orientation angles of 25° and 28° as compared to 11° in the thylakoids). This decrease in orientation might be due to an alteration in the binding of these pigments, caused by the purification procedure. In *Mantoniella*, especially β-carotene and the xanthophylls of the xanthophyll cycle have been reported to be sensitive to detergent treatment and sucrose gradient centrifugation [16].

4. Discussion

This study has provided the first CD and LD data on Chl *a*/*b*/*c*-containing membranes and the purified LHC. The organization of the pigment molecules in *M. squamata* is of special interest, because the prasinophycean algae most probably represent an ancient group of the chlorophyta, and data on this molecular organization may therefore provide important information on the evolution of the Chl-binding proteins.

At the organizational level, intact cells of *Mantoniella* exhibited much weaker anomalous CD signals than those observed for higher plant chloroplasts, and no anomalous CD could be identified for the isolated thylakoids and purified LHC. Anomalous CD (intense bands combined with differential scattering of the left and right circularly polarized light) has been observed in the granal thylakoids and lamellar aggregates of isolated LHC II and demonstrated to originate from the chiral macroorganization of the chromophores, features typical of psi-type (polymer or salt-induced) aggregates (cf. [18]). The fact that this type of signal is weak in the case of *Mantoniella* cells and absent in the case of isolated thylakoids can be explained by the organization of the thylakoid membranes in the chloroplasts of prasinophycean algae, which is clearly different from that in higher plants. In *Mantoniella*, the thylakoid membranes are aligned parallel to each other, with spacings of 2–8 nm [1]. This regular membrane organization, which cannot be maintained when the cells (and the chloroplasts) are broken, can give rise to the weak anomalous CD observed in intact cells.

The absence of a large CD for the Chl *a*/*b*/*c* LHC could point to a different role of the LHC of *Mantoniella* in the organization of the thylakoid ultrastructure as compared to LHC II. In higher plants, the extensive self-aggregation capability of LHC II has been shown to play a key role in the stacking of the membranes (cf. [39,40]) and also in the lateral organization, i.e. the packing of the PS II particles and the sorting of PS II and PS I between the grana and stroma membranes, respectively [32,33]. As concerns the CD of the purified LHC, it must be stressed that the procedure for the isolation of Chl *a*/*b*/*c* antenna complexes does not favor the formation of lamellar aggregates, which are associated with psi-type CD bands (cf. [41]).

The CD signature of the thylakoid membranes and purified LHC of *Mantoniella* to a considerable extent resembles those of higher plant thylakoids suspended in a low-salt hypotonic medium (cf. [32]) and of non-aggregated LHC II (cf. [31]). This was most clearly indicated by the similarity of the band structures of the Chl *a*/*b*/*c* and Chl *a*/*b* LHCs between 640 and 700 nm. This suggests that the organization of Chls *a* and *b* in the Chl *a*/*b*/*c* LHC is similar to that in LHC II. However, as revealed by the positive LD of Chl *b* bands in both the thylakoids and the LHC, this is not true for the binding of the Chl *b* molecules, which in *Mantoniella* seem to be preferentially oriented in the plane of the membrane (Table 1). In LHC II, the Chl *b* dipoles have been shown to possess an out of plane orientation [30], close to the magic angle [35].

An even more significant difference was revealed as concerns the interactions involving the B_X dipoles of Chl *a*, revealed by the intense (+) 441 nm CD band. This band is about one order of magnitude higher than similar bands in LHC II and Chl *a*/*c* LHCs. Further, it is accompanied by intense negative bands between 465 and 510 nm. Hence, it is proposed that the bands between 440 and 510 nm originate from intense interactions between Chl and carotenoid dipoles of higher excited states.

As regards the Chl *c* molecules, the CD band at (−) 630 nm points to the fact that these pigments possess significant optical activity in the *Mantoniella* LHC, this appearing to be stronger than in the Chl

a/*c* LHCs of *P. meiringensis*, *D. dichotoma* and *Amphidinium carterae* [23,36,42]. It is also noteworthy that the Chl *c* molecules in *Mantoniella* are oriented with their Q_Y dipoles lying close to the membrane plane (orientation angle 29°); a similar orientation of Chl *c* has been described for two chromophytic algae [21]. In the LHC of *Mantoniella*, it has been proposed that the binding of Chl *c* molecules takes place on the luminal half of helix I, which contains a stretch of polar and charged amino acids [4]. This domain, which is absent from LHC II, could provide the suitable environment needed for the binding of Chl *c* molecules, which are more polar than Chl *a* and *b*. In Chl *a*/*c*-containing LHCs, where Chl *b* is replaced by Chl *c*, the Chl *c* binding is thought to occur on helix II; this displays the highest degree of divergence in the different LHC proteins [43] and in higher plants contains the binding sites for three of the five Chl *b* molecules of LHC II [17]. However, Chl *c* binding in the LHC of *Mantoniella* is most probably different from that in the Chl *a*/*c* LHCs, because the *Mantoniella* species (apart from Chl *a* and *c*) also binds Chl *b* in even higher amounts than LHC II (six Chl *b* as compared to five in higher plants) [15]. A comparison of the amino acid sequences of the LHC proteins in *Mantoniella* and higher plants reveals that especially those amino acids that are proposed to serve as ligands to the Chl *a* and *b* molecules (His, Asn, Gln) are highly conserved [4]. These results suggest that in *Mantoniella*, most of the Chl *a* and Chl *b* molecules are bound to the same ligands as in LHC II. However, other factors must also influence the binding of the Chl *b* molecules, which, unlike the situation in LHC II, display an in the plane orientation in *Mantoniella*.

In the thylakoids and LHC of *Mantoniella*, the carotenoids also exhibit a significant degree of orientation, as can be seen from the positive LD bands between 490 and 520 nm. This is in contrast with the Chl *a*/*c*-containing thylakoids and LHC of *P. meiringensis*, which exhibit negative LD in the region of the carotenoids; tilting out of the dipoles from the membrane plane has therefore been proposed [23]. The major light-harvesting xanthophyll of the diatoms, fucoxanthin, is considered to be oriented at an angle $<35°$ with respect to the plane of the thylakoid membrane in *P. lutherii* and *P. tricornutum* [21]. A similar orientation of fucoxanthin has been observed for thylakoids and the Chl *a*/*c* fucoxanthin complex of the diatom *C. fusiformis* [22]. Earlier studies on the thylakoids and LHC II established that some carotenoids in higher plants likewise exhibit a high degree of orientation and lie close to the plane of the membrane, with the optical transitions polarized along the polyenic chain [44,45]. Recent results, taking into account the structure of LHC II, have helped to clarify the orientation of the xanthophyll molecules. A monomeric LHC II unit contains two lutein molecules forming the cross-brace essential for the tertiary structure of the LHC II protein [17]. The lutein molecules are perpendicular to each other and to the axis that connects the centers of two molecules. Crystallographic data [17] allow a prediction of an out of plane orientation for these carotenoids, as indeed derived from polarized fluorescence excitation spectra by Gruszecki et al. [46]. In this study, neoxanthin was found to adopt a similar orientation to lutein, whereas violaxanthin was suggested to exhibit a planar orientation with respect to the plane of the thylakoid membrane.

In *Mantoniella*, the strong positive LD signal at 520 nm is most probably due to prasinoxanthin. Although prasinoxanthin is bound to the LHC protein in the same stoichiometric amounts as lutein in LHC II (two molecules per polypeptide) [15], there is no experimental evidence for a cross-brace arrangement of the two molecules. However, this does not necessarily mean the absence of such a structure in LHC. The present data strongly suggest that the carotenoid molecules absorbing at 520 nm are arranged as proposed for violaxanthin in the trimeric LHC II [46]. In other regions, the interpretation of the carotenoid LD is complicated by the presence of nine different xanthophylls with very similar absorption spectra.

In summary, we conclude that the pigment organization in *M. squamata* exhibits some typical features of Chl *a*/*b*-containing organisms. The resemblance of the pigment organization to that in other Chl *c*-containing membranes and Chl *a*/*c* LHCs appears to be more limited. This is in good agreement with the structural data from amino acid analysis, which reveal a closer relationship of the *Mantoniella* LHC to the Chl *a*/*b*-binding LHCs of higher plants and green algae [4,7]. On the other hand, it must also be emphasized that there are very significant differ-

ences between the Chl *a*/*b* and Chl *a*/*b*/*c* complexes, which cannot be accounted for simply in terms of the different pigment compositions, but are rather due to differences in the architecture of the LHCs and interactions between different chromophores.

Acknowledgements

Financial support from the Deutsche Forschungsgemeinschaft and from OTKA (Hungarian Fund for Basic Research) Grants T019226 and T030324 is gratefully acknowledged. The authors express their thanks to Prof. Dr. A. Scherz for fruitful discussions.

References

[1] P. Krämer, C. Wilhelm, A. Wild, E. Mörschel, E. Rhiel, Ultrastructure and freeze-fracture studies of thylakoids of *Mantoniella squamata* (Prasinophyceae), Protoplasma 147 (1988) 170–177.

[2] A. Schmitt, A. Herold, C. Welte, A. Wild, C. Wilhelm, The light-harvesting system of the unicellular alga *Mantoniella squamata* (Prasinophyceae): Evidence for the lack of a Photosystem I specific antenna complex, Photochem. Photobiol. 57 (1993) 132–138.

[3] B.R. Green, D.G. Durnford, The chlorophyll-carotenoid proteins of oxygenic photosynthesis, Annu. Rev. Plant Physiol. Plant Mol. Biol. 47 (1996) 685–714.

[4] E. Rhiel, E. Mörschel, The atypical chlorophyll *a*/*b*/*c* light-harvesting complex of *Mantoniella squamata*: molecular cloning and sequence analysis, Mol. Gen. Genet. 240 (1993) 403–413.

[5] C. Passaquet, J.C. Thomas, L. Caron, N. Hauswirth, F. Puel, C. Berkaloff, Light-harvesting complexes of brown algae. Biochemical caracterization and immunological relationships, FEBS Lett. 280 (1991) 21–26.

[6] M.W. Fawley, K.D. Stewart, K.R. Mattox, The novel light-harvesting pigment-protein complex of *Mantoniella squamata* (Chlorophyta): phylogenetic implications, J. Mol. Evol. 23 (1986) 168–176.

[7] A. Schmitt, G. Frank, P. James, W. Staudenmann, H. Zuber, C. Wilhelm, Polypeptide sequence of the chlorophyll *a*/*b*/*c*-binding protein of the prasinophycean alga *Mantoniella squamata*, Photosynth. Res. 40 (1994) 269–277.

[8] E. Pichersky, R. Subramaniam, M.J. White, J. Reid, R. Aebersold, B.R. Green, Chlorophyll *a*/*b* binding (CAP) polypeptides of CP 29, the internal chlorophyll *a*/*b* complex of PS II: characterization of the tomato gene encoding the 26 kDa (type 1) polypeptide, and evidence for a second CP 29 polypeptide, Mol. Gen. Genet. 227 (1991) 277–284.

[9] B.R. Green, E. Pichersky, K. Kloppstech, Chlorophyll *a*/*b*-binding proteins: an extended family, Trends Biochem. Sci. 16 (1991) 181–186.

[10] A. Grossman, A. Manodori, D. Snyder, Light-harvesting proteins of diatoms: Their relationship to the chlorophyll *a*/*b* binding proteins of higher plants and their mode of transport into plastids, Mol. Gen. Genet. 224 (1990) 91–100.

[11] R.G. Hiller, J.M. Anderson and A.W.D. Larkum, The chlorophyll-protein complexes of algae, in: H. Scheer (Ed.), Chlorophylls, CRC Press, Boca Raton, FL, 1991, pp. 530–547.

[12] C. Büchel, C. Wilhelm, Isolation and characterization of a photosystem I-associated antenna (LHC I) and a photosystem I-core complex from the chlorophyll *c*-containing alga *Pleurochloris meiringensis*, J. Photochem. Photobiol. 20 (1993) 87–93.

[13] J. Knoetzel, L. Rensing, Characterization of the photosynthetic apparatus from the marine dinoflagellate *Gonyaulax polyedra*. I. Pigment and polypeptide composition of the pigment protein complexes, J. Plant Physiol. 136 (1990) 271–279.

[14] K.S. Rowan, Photosynthetic Pigments of Algae, Cambridge University Press, Cambridge, 1989.

[15] C. Wilhelm, S. Kolz, M. Meyer, A. Schmitt, H. Zuber, E.S. Egeland, S. Liaaen-Jensen, Refined carotenoid analysis of the major light-harvesting complex of *Mantoniella squamata*, Photosynthetica 33 (1997) 161–171.

[16] R. Goss and C. Wilhelm, In *Mantoniella squamata* deepoxidized xanthophyll cycle pigments show a tighter pigment protein coupling than the respective expoxidized forms, in: G. Garab (Ed.), Photosynthesis: Mechanisms and Effects, Vol. III, Kluwer Academic Publishers, 1998, pp. 2317–2320.

[17] W. Kühlbrandt, D.N. Wang, Y. Fujiyoshi, Atomic model of plant light harvesting complex by electron crystallography, Nature 367 (1994) 614–621.

[18] G. Garab, Linear and circular dichroism, in: J. Amesz and A.J. Hoff (Eds.), Biophysical Techniques in Photosynthesis, Kluwer Academic Publishers, Amsterdam, 1996, pp. 11–40.

[19] J. Breton and A. Vermeglio, Orientation of photosynthetic pigments in vivo, in: Govindjee (Ed.), Photosynthesis, Academic Press, New York, 1982, pp. 153–193.

[20] R.G. Hiller, C.D. Scaramuzzi, J. Breton, The organisation of photosynthetic pigments in a cryptophyte alga: A linear dichroism study, Biochim. Biophys. Acta 1102 (1992) 360–364.

[21] R.G. Hiller, J. Breton, A linear dichroism study of photosynthetic pigment organisation in two fucoxanthin-containing algae, Biochim. Biophys. Acta 1102 (1992) 365–370.

[22] B.-D. Hsu, J.-Y. Lee, Orientation of pigments and pigment-protein complexes in the diatom *Cylindrotheca fusiformis*. A linear dichroism study, Biochim. Biophys. Acta 893 (1987) 572–577.

[23] C. Büchel, G. Garab, Organization of the pigment molecules in the chlorophyll *a*/*c* light-harvesting complex of *Pleurochloris meiringensis* (Xanthophyceae). Characterization with circular dichroism and absorbance spectroscopy, J. Photochem. Photobiol. B Biol. 37 (1997) 118–124.

[24] C. Büchel, G. Garab, Organization of the pigment molecules

in the thylakoids and the chlorophyll *a/c* light-harvesting complex of a xanthophyte alga, *Pleurochloris meiringensis*. A linear dichroism study, J. Photochem. Photobiol. B Biol. 44 (1998) 199–204.
[25] D. Müller, Über jahres- und lunarperiodische Erscheinungen bei einigen Braunalgen, Bot. Mar. 4 (1962) 140–155.
[26] R. Ziegler, K. Egle, Zur quantitativen Analyse der Chloroplastenpigmente. I. Kritische Überprüfung der spektralphotometrischen Chlorophyll-Bestimmung, Beitr. Biol. Pflanzen 41 (1965) 11–37.
[27] I. Abdourakhmanov, A.O. Ganago, Y.E. Erokhin, A. Solovev, V. Chugunov, Orientation and linear dichroism of the reaction centers from *Rhodopseudomonas sphaeroides* R-26, Biochim. Biophys. Acta 546 (1979) 183–186.
[28] B. Hecks, C. Wilhelm, H.-W. Trissl, Functional organization of the photosynthetic apparatus of the primitive alga *Mantoniella squamata*, Biochim. Biophys. Acta 1274 (1996) 21–30.
[29] R.L. van Metter, Excitation energy transfer in the light-harvesting chlorophyll *a/b* protein, Biochim. Biophys. Acta 462 (1977) 642–658.
[30] P.W. Hemelrijk, S.L.S. Kwa, R. van Grondelle, J.P. Dekker, Spectroscopic properties of LHC II, the main light-harvesting chlorophyll *a/b* protein complex from chloroplast membranes, Biochim. Biophys. Acta 1098 (1992) 159–166.
[31] D. Gülen, R.S. Knox, Absorption and circular dichroism of the chlorophyll-protein CP II: Extension of a trimeric exciton model, Photobiochem. Photobiophys. 7 (1984) 277–286.
[32] G. Garab, J. Kieleczawa, J.C. Sutherland, C. Bustamante, G. Hind, Organization of pigment-protein complexes into macrodomains in the thylakoid membranes of wild type and chlorophyll *b*-less mutant of barley as revealed by circular dichroism, Photochem. Photobiol. 54 (1991) 273–281.
[33] V. Barzda, L. Mustardy, G. Garab, Size dependency of circular dichroism in macroaggregates of photosynthetic pigment-protein complexes, Biochemistry 33 (1994) 10837–10841.
[34] H. Kramer, J. Amesz, Anisotropy of the emission and absorption bands of spinach chloroplasts measured by fluorescence polarization and polarized excitation spectra at low temperature, Biochim. Biophys. Acta 682 (1982) 201–207.
[35] H. van Amerongen, L.S. Kwa, M. Bauke, M. van Bolhuis, R. van Grondelle, Polarized fluorescence and absorption of macroscopically aligned light harvesting complex II, Biophys. J. 67 (1994) 837–847.
[36] M. Mimuro, T. Katoh, H. Kawai, Spatial arrangement of pigments and their interaction in the fucoxanthin-chlorophyll *a/c* protein assembly (FCPA) isolated from the brown alga *Dictyota dichotoma*. Analysis by means of polarized spectroscopy, Biochim. Biophys. Acta 1015 (1990) 450–456.
[37] M.A.M.J. van Zandvoort, D. Wrobel, P. Lettinga, G. van Ginkel, Y.K. Levine, The orientation of the transition dipole moments of chlorophyll a and pheophytin a in their molecular frame, Photochem. Photobiol. 62 (1995) 299–308.
[38] E. Rhiel, W. Lange, E. Mörschel, The unusual light-harvesting complex of *Mantoniella squamata*: supramolecular composition and assembly, Biochim. Biophys. Acta 1143 (1993) 163–172.
[39] L.A. Staehelin, C.J. Arntzen, Regulation of chloroplast membrane function: Protein phosphorylation changes the spatial organization of membrane components, J. Cell Biol. 97 (1983) 1327–1337.
[40] J. Barber, Surface electric charges and protein phosphorylation, in: L.A. Staehelin and C.J. Arntzen (Eds.), Encyclopedia of Plant Physiology, Vol. 19, Springer Verlag, Berlin, 1986, pp. 653–664.
[41] I. Simidjiev, V. Barzda, L. Mustardy, G. Garab, Isolation of lamellar aggregates of the light-harvesting chlorophyll *a/b* protein complex of Photosystem II with long-range chiral order and structural flexibility, Anal. Biochem. 250 (1997) 169–175.
[42] R.G. Hiller, P.M. Wrench, A.P. Gooley, G. Shoebridge, J. Breton, The major intrinsic light-harvesting protein of *Amphidinium*: Characterization and relation to other light-harvesting proteins, Photochem. Photobiol. 57 (1993) 125–131.
[43] B.R. Green, E. Pichersky, Hypothesis for the evolution of three-helix Chl *a/b* and Chl *a/c* light-harvesting antenna proteins from two-helix and four-helix ancestors, Photosynth. Res. 39 (1994) 149–162.
[44] J. Breton, M. Michel-Villaz, G. Paillotin, Orientation of pigments and structural proteins in the photosynthetic membrane of spinach chloroplasts: A linear dichroism study, Biochim. Biophys. Acta 314 (1973) 42–56.
[45] P. Haworth, C.J. Arntzen, P. Tapie, J. Breton, Orientation of pigments in the thylakoid membrane and in the isolated chlorophyll-protein complexes of higher plants. I. Determination of optimal conditions for linear dichroism measurement, Biochim. Biophys. Acta 679 (1982) 428–435.
[46] W.I. Gruszecki, W. Grudzinski, A. Banaszek-Glos, M. Matula, P. Kernen, Z. Krupa, J. Sielewiesiuk, Xanthophyll pigments in light-harvesting complex II in monomolecular layers: localisation, energy transfer and orientation, Biochim. Biophys. Acta 1412 (1999) 173–183.