



Chlorophyll triplet quenching by fucoxanthin in the fucoxanthin–chlorophyll protein from the diatom *Cyclotella meneghiniana*

Marilena Di Valentin^a, Claudia Büchel^b, Giorgio Mario Giacometti^c, Donatella Carbonera^{a,*}

^a Dipartimento di Scienze Chimiche, Università di Padova, via Marzolo 1, 35131 Padova, Italy

^b Institute of Molecular Biosciences, Goethe University Frankfurt, 60438 Frankfurt, Germany

^c Dipartimento di Biologia, Università di Padova, via U. Bassi 58/B, 35121 Padova, Italy

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ABSTRACT

In this work we present an optically detected magnetic resonance (ODMR) study on the triplet states populated under illumination in the isolated fucoxanthin–chlorophyll light-harvesting complex from the diatom *Cyclotella meneghiniana*. Evidence for the quenching of chlorophyll triplet states by fucoxanthin is provided, showing that this carotenoid is able to perform the photoprotective role. For the first time, the magnetic parameters characterizing the fucoxanthin triplet state have been determined.

The results reveal analogies but also differences with respect to the triplet–triplet energy transfer process, which involves chlorophylls *a* and carotenoids in the LHC complex from dinoflagellates and LHCII from higher plants. The degree of efficiency of the photoprotection mechanism, in these light harvesting complexes, is discussed in terms of pigment–protein structure.

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1. Introduction

The photosynthetic apparatus of eukaryotic diatom algae is believed to be very similar to other eukaryotic thylakoid systems, with essentially the same light-induced reactions and electron transport chain. However, compared to other systems, the details of the molecular mechanisms of the photosynthetic process are not known at the same extent.

The antenna proteins of diatoms are related to the *cab* proteins of higher plants [1–3]. The homologies are significant, however, the polypeptides are smaller in size (17–23 kDa) and the proteins are expected to be more hydrophobic. The antenna proteins bind chlorophyll *a* (Chl *a*) like all *cab*-proteins, with some of the Chl *a* binding sites conserved in the structure. Chl *b* is replaced by Chl *c*, as in LHC from dinoflagellates. Because fucoxanthin is the main carotenoid present in these antenna proteins, they are usually called fucoxanthin–chlorophyll proteins (FCP). In contrast to the LHCII of higher plants, which binds 14 chlorophylls and 4 carotenoids [4], the FCP complex binds more carotenoids and less Chls. Different stoichiometries have been proposed up to now ranging from a 4:4:1 to a 8:8:2 fucoxanthin/Chl-*a*/Chl-*c*₂ composition per

monomer [5–6]. The high amount of fucoxanthin improves capturing of green light which is available in aquatic environments. Another carotenoid, diadinoxanthin which can be converted to diatoxanthin that is involved in the non-photochemical quenching (NPQ), is present only in trace amounts [5]. FCPs were isolated in different oligomeric states from *Cyclotella meneghiniana*, with a preferential organization of the 18 kDa proteins into trimers and of the 19 kDa polypeptides into higher oligomeric states [7]. This marks a difference in the supramolecular structure of the FCP proteins compared to LHCII, likely reflecting the different arrangement of the thylakoid membranes in diatoms, which lack the grana–stroma distinction [7]. The different pigment content is reflected in the energy transfer (ET). In LHCII, where Chl *a* and Chl *b* are the main pigments, the ET from carotenoids to Chl *a*, which is 80% efficient, proceeds mainly from S₂ and to some extent also from the S₁ states [8–10]. It has been shown that for PCP, which like FCP contains high amounts of a carbonyl-containing carotenoid, the dominant ET pathways involve the lower excited states of peridinin [11,12]. A transient absorption measurement on FCP has shown efficient energy transfer to Chl *a*, but it has also pointed out the presence of a fraction of carotenoids that is not transferring energy to Chl *a* [13]. Gildenhoff et al. [14] determined lifetimes for FCPs of <150 fs for the fucoxanthin S₂ state (transferring to the Q_x of Chl *a*), and 0.6/0.9 ps for the unrelaxed and 2.6/4.2 ps for the relaxed S₁/ICT state (transferring to Q_y), whereby the higher values represent the lifetimes of the ‘red’ fucoxanthin (Fx) molecules, absorbing at slightly longer wavelength and the lower ones those of the ‘blue’ Fx. Using anisotropy measurements it was concluded

Abbreviations: FCP, fucoxanthin chlorophyll protein; Fx, fucoxanthin; Car, carotenoid; Chl, chlorophyll; ODMR, optically detected magnetic resonance; FDMR, fluorescence detected magnetic resonance; ADMR, absorption detected magnetic resonance; T–S, triplet minus singlet.

* Corresponding author. Fax: +39 498275161.

E-mail address: donatella.carbonera@unipd.it (D. Carbonera).

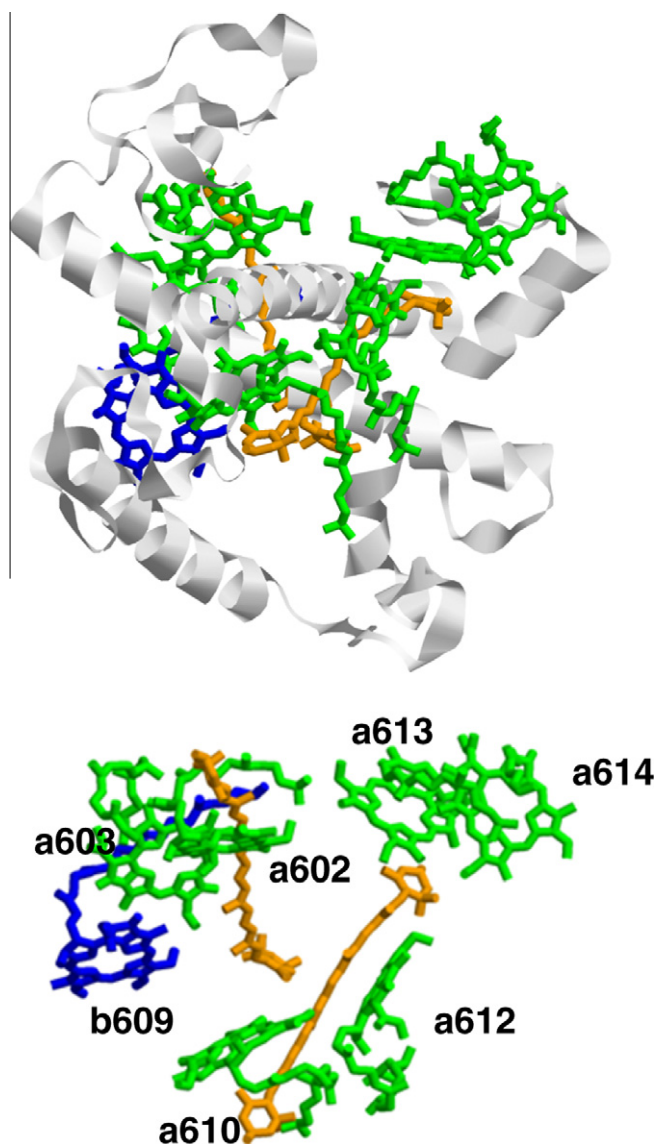


Fig. 1. Structural models. *Top*: structure of the pigments associated with the core basic unit of the LHCII complex derived from 1RWV PDB code. The protein backbone and the Chl *b* (blue) and Chl *a* (green) molecules around the carotenoids (orange) in L1 and L2 sites are shown. *Bottom*: conserved chlorophyll and carotenoid binding sites in FCP based on the sequence alignment with the 3D structure of LHCII. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

that one Fx-red and two of the Fxs-blue/green transfer their energy directly to Chl *a*, whereas a further Fx-blue/green is depending on another Fx molecule for excitation energy transfer to Chl *a* [15].

The lack of spectral evolution in the Chl *a* bleach band upon excitation and the fact that it is centered at 670 nm, suggests that the Chls in the FCP complex are not excitonically coupled as they appear to be in LHCII [13]. The fluorescence-excitation measurements have demonstrated that the energy transfer from Chl *c* to Chl *a*, occurs on a timescale of a 100 fs and is 100% efficient [13].

Carotenoids are known to be involved in photoprotection of photosynthetic systems, not only in NPQ [16–18] but also as direct quenchers of the Chl *a* triplets ($^1\text{Chl } a$) in the light harvesting complexes. In this way they prevent the formation of singlet oxygen and subsequent harmful oxidation [19].

In the past we have studied the formation of Car triplet states (^1Car) in LHCII, LHC and PCP by means of several spectroscopic

techniques [20–25] and computational methods [26]. Carotenoids carrying the triplet states have been characterized, showing specific interactions with Chls [20–28]. Analogies and differences among the LHCs have been pointed out. In LHCII, Chl *a* 612/603 [4] were identified as the sites having the highest probability of forming triplet states which are further quenched by the two central luteins [23,29]. In PCP, Per614 [30] was recognized as the photoprotective carotenoid. Our investigation on the quenching mechanism of ^1Chl states in isolated LHC from *Amphidinium carterae* has revealed more similarities with LHCII rather than with the peripheral PCP. Considering the high homology of LHC to LHCII and deriving a structural model for the core of the LHC protein, we have found that the carotenoids responsible for the triplet quenching could be two peridins located in buried sites, in strict analogy with the L1 and L2 of LHCII. However, EPR spectra analysis has highlighted some differences between LHC and LHCII, leading to the conclusion that, if the structural model is correct, most probably in LHC the sites of ^1Chl formation are Chl *a* 602 and Chl *a* 610, instead of Chl *a* 603 and Chl *a* 612 as in LHCII.

Recently, a structural model for FCP based on the homology with LHCII, showing six conserved Chl *a*/Chl *b* ligands around the two central carotenoids L1, L2 binding sites, has been proposed [6] (Fig. 1).

In view of the similarities, but also of the differences, between the light harvesting systems of higher plants, dinoflagellates and diatoms, it is interesting to extend the study of the mechanism of ^1Chl quenching in the FCP complex.

In this work we have performed both fluorescence detected magnetic resonance (FDMR) and absorption detected magnetic resonance (ADMAR) to characterize the triplet states populated in FCP and compared the results with those previously obtained in LHCII and LHC.

2. Materials and methods

2.1. Sample preparation

FCP complexes from *C. meneghiniana* were prepared according to the method described in [7]. In brief, thylakoids were solubilised with β -dodecyl maltoside and complexes were separated by sucrose gradient centrifugation. The lower brown band was harvested and concentrated to an OD of 85 at 440 nm. The samples used for measurements represented a mixture of FCPa and FCPb complexes, thus having the same pigment composition as the complexes described in [13].

For all the experiments, the FCP samples were dissolved in buffer (6% sucrose, 10 mM Mes pH 6.5, 2 mM KCl, glycerol (60% v/v)).

2.2. ODMR and emission spectra

Experiments were performed in apparatus described elsewhere [31,32].

The principle of the ODMR technique may be briefly described as follows: under continuous illumination a steady-state population is generated in the three triplet spin-sublevels. Application of a resonant microwave field between a couple of spin-sublevels induces a change of the steady-state population of the triplet state, due to the anisotropy of the decay and population rates of the three spin-sublevels. The change of population may be detected as a corresponding change of the emission and/or absorption of the system [33,34].

In the FDMR experiments the fluorescence, excited by a halogen lamp (250 W) focused into the sample and filtered by 5 cm-length solution of CuSO_4 1 M, was collected through appropriate band-pass filters, by a photodiode. Low temperature emission spectra

were detected in the same apparatus used for FDMR experiments, using the same excitation source, but substituting the band-pass filters, before the detector, by a monochromator.

In the ADMR experiments, the same excitation lamp was used but without filters before the sample, except for 5 cm-length water and heat filters. The beam was focused into a monochromator after passing the sample and finally collected by a photodiode. By fixing the microwave frequency at a resonant value, while sweeping the detection wavelength, microwave induced triplet-minus-singlet (T–S) spectra were registered.

The temperature of all the experiments was 1.8 K. At this temperature spin–lattice relaxation is inhibited and the ODMR signals are detectable.

3. Results

The room temperature absorption spectrum of FCP (data not shown) is identical to that previously reported [6]. The Chl *a* Q_y band is centered at 672 nm. In the Soret region, there are two bands due to Chl *a* (441 nm) and Chl *c*₂ (457 nm). Carotenoid absorption bands overlap with the Soret bands of Chls and extend further to the red.

The emission spectrum of FCP obtained at 1.8 K, is centered at 676.5 nm, see Fig. 2. No emission bands due to Chl *c*₂ molecules have been detected (range not shown).

Illumination of the samples at 1.8 K induces the formation of ^TCar which can be detected by monitoring the Chl *a* fluorescence of the sample while sweeping the microwave field, as previously reported for LHCII, LHC and PCP [27,35,22]. Although carotenoids are non-fluorescing molecules, a change of the steady-state population of the ^TCar states, induced by a resonant microwave field, is reflected by a change of the intensity of the emission of the nearby Chl molecules, from which they are populated [32]. The FDMR spectra, detected in the microwave field region where the $2|E|$, the $|D| - |E|$, and the $|D| + |E|$ transitions of ^TCar are expected, are shown in Fig. 3A (*D* and *E* represent the zero field splitting parameters (ZFS) of the triplet state). The $|D| - |E|$ transitions are hardly visible. The $|D| + |E|$ transitions show the contribution of two triplet components, indicating that ^TChl states in the FCP can be quenched by populating two slightly different ^TCar populations. The relative contribution of the two different ^TCar populations (T1: $|D| = 1263 \pm 10$ MHz, $|E| = 122 \pm 2$ MHz; T2: $|D| = 1307 \pm 10$ MHz, $|E| = 122$ MHz) to the FDMR spectrum is independent on the

detection wavelength in the emission spectrum, as shown in Fig. 3 where the detail of the $|D| + |E|$ transitions detected at different wavelengths is shown.

Since it is known that ^TCars are not populated directly from their excited singlet states [19], their presence upon illumination of FCP must be due to ^TChl quenching. Illumination of the complex at low temperature results also in the production of ^TChl states, which are not quenched by carotenoids. The spectral region of FDMR spectra, where the $|D| - |E|$ and the $|D| + |E|$ transitions of ^TChl *a* states are expected [32], is shown in Fig. 3B. The $2|E|$ transition is too weak to be detected, as usually found for ^TChl states. The ^TChl states of FCP show a dependence on the detection wavelength and are characterized by values $|D| = 855\text{--}864$ MHz and $|E| = 118 \pm 2$ MHz.

To better characterize the Car and Chl molecules carrying the triplet states, the microwave-induced T–S spectra were taken by setting the microwave field at different resonant frequencies. The results are shown in Fig. 4.

From the carotenoid T–S spectra the position of the intense T–T absorption band, which is the main (positive) feature in the T–S spectrum of carotenoids and which always appears to the red side of the red-most $S_0 \rightarrow S_2$ (negative) absorption band, can be determined. This band is centered at 574 nm. Unfortunately the low intensity of the signals does not permit to detect the T–S spectra by setting different resonant frequencies in the $|D| + |E|$ transitions, where the two triplet components are resolved in the FDMR spectra. However, by setting the $2|E|$ transitions at a frequency centered at 247 and 230 MHz, which should allow to excite preferentially the two different triplet states, the same spectrum is obtained (not shown), meaning that the two triplet states belong to carotenoid molecules having very similar optical properties. The T–T absorption bands partially overlap with the corresponding bleaching bands of the singlet–singlet absorption ($S_0 \rightarrow S_2$), whose $0 \rightarrow 0$ transitions are around 510–535 nm.

In the Chl Q_y absorption region of the T–S of ^TCar, an additional weak negative band has also been detected, at 669 nm (Fig. 4). This band is due to the interaction between the Car molecule carrying the triplet state and the nearby Chls which “feel” the change of the electronic state of the Car. Similar bands have been reported in the past for several light-harvesting complexes [22,27,36,37].

Together with the ^TCar states, a consistent population of unquenched ^TChl *a* is also produced in the isolated FCP upon photo-excitation, meaning that the efficiency of the quenching by carotenoids does not amount to 100%, at least at the low temperature of the experiments. In the Chl T–S spectrum associated to the maximum of the $|D| + |E|$ transitions, at 986 and 974 MHz, the absorption of the Chl *a* molecules giving the triplet states is seen as a bleaching at 674–676 nm (Fig. 4). These wavelengths indicate that the unquenched ^TChl states belong to the “red most” Chls of the complex.

No Chl *c* triplet states have been detected either by FDMR or by ADMR.

4. Discussion

The spectroscopic results obtained for FCP are very similar to those previously obtained for the LHC from dinoflagellates. As in that case, the triplet–triplet ET between Chl and Car is active but, despite the high Car/Chl, the efficiency of the ^TChl *a* quenching is low. In fact, a large population of unquenched ^TChl *a* is present. Low amount of unquenched ^TChl *a* were also detected before in isolated LHCII [18], as well as in thylakoids [31]. The incomplete quenching of ^TChl *a* in FCP could be compensated in vivo by the direct scavenging of singlet oxygen in an environment which is rich in carotenoids.

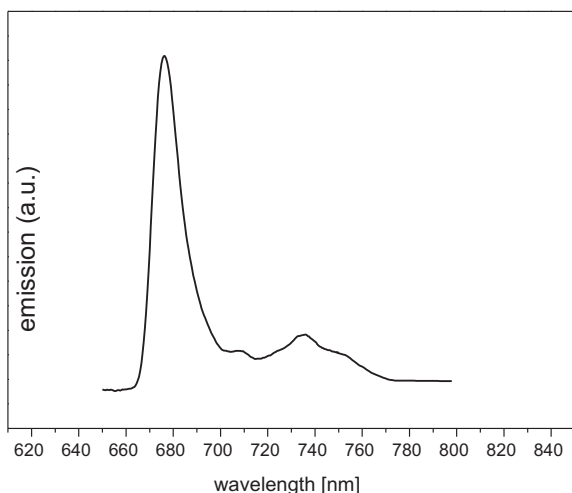


Fig. 2. Emission spectrum of the FCP at 1.8 K.

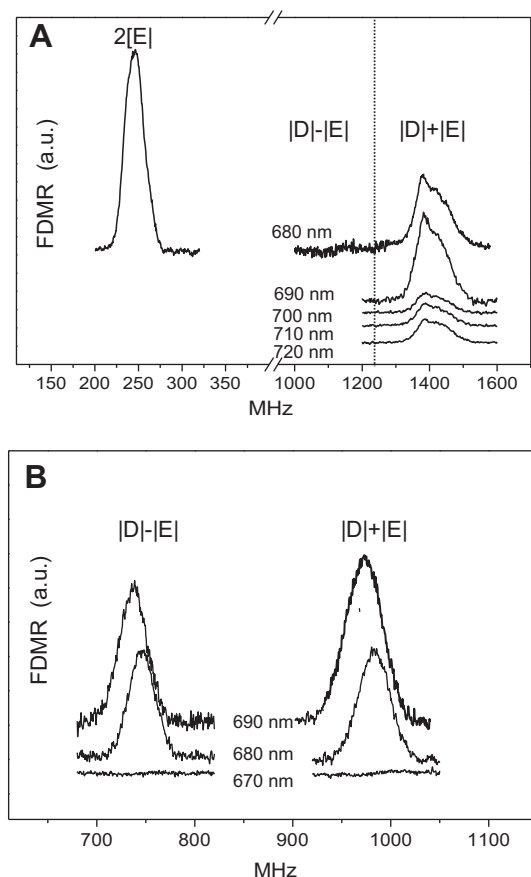


Fig. 3. FDMR spectra of FCP, detected at different wavelengths at 1.8 K. (A) ^1Car : $2|E\rangle$, the $|D\rangle - |E\rangle$, and the $|D\rangle + |E\rangle$ transitions. Ampl. mod. frequency, 315 Hz; tc, 600 ms. $2|E\rangle$ transition divided by a factor of six. (B) ^1Chl : $|D\rangle - |E\rangle$, and the $|D\rangle + |E\rangle$ transitions. Ampl. mod. frequency, 33 Hz; tc, 600 ms.

ODMR data show that two slightly different ^1Car states are present in the sample. The same results have been obtained before in both LHC and LHCII. The two ^1Car states were assigned to the Cars located in the L1 and L2 sites of the LHCII structure, having specific environments and, consequently, slightly different ZFS parameters. Recently Resonance Raman spectroscopy has been used to characterize the structures and environments of fucoxanthin in FCP [5]. Two Fxblue's were proposed to occupy L1 and L2 sites. However, our results point towards Fxrgreen's or Fxred's as candidate for these occupancies. In fact, the T–S spectra associated to the ^1Car states in FCP show that the fucoxanthin molecules involved in the quenching of $^1\text{Chl } a$ are the low-energy ones present in the complex, absorbing at about 510–535 nm.

In the $\text{Chl } Q_y$ absorption region of the ^1Car T–S spectra, it is possible to distinguish a bleaching at 669 nm. This means that $^1\text{Cars}$ are very close to $\text{Chl } a$ absorbing at that wavelength. No bleaching of $\text{Chl } c_2$ is present, making a difference with respect to both LHC and LHCII complexes. Those were characterized, not only by $\text{Chl } a$ but also by $\text{Chl } c_2$ and $\text{Chl } b$ interaction bands, respectively, in the T–S spectra associated to the peridinin/lutein triplet states [27,22]. The lack of the $\text{Chl } c_2$ bleaching in the spectrum of FCP probably means that the one or two $\text{Chl } c$ molecule/s which is/are present in the FCP monomer, is/are not located at interacting distance with the carotenoid where the triplet states become localized. The proposed structural model for FCP [31], shown in Fig. 1, based on six conserved ligands of $\text{Chl } a$ plus a $\text{Chl } b$ and on two conserved central binding sites of carotenoids of LHCII, suggests that, also in FCP, the L1 and L2 sites may well be the sites of ^1Chl quenching by Car. However, the absence of the bleaching of Chl

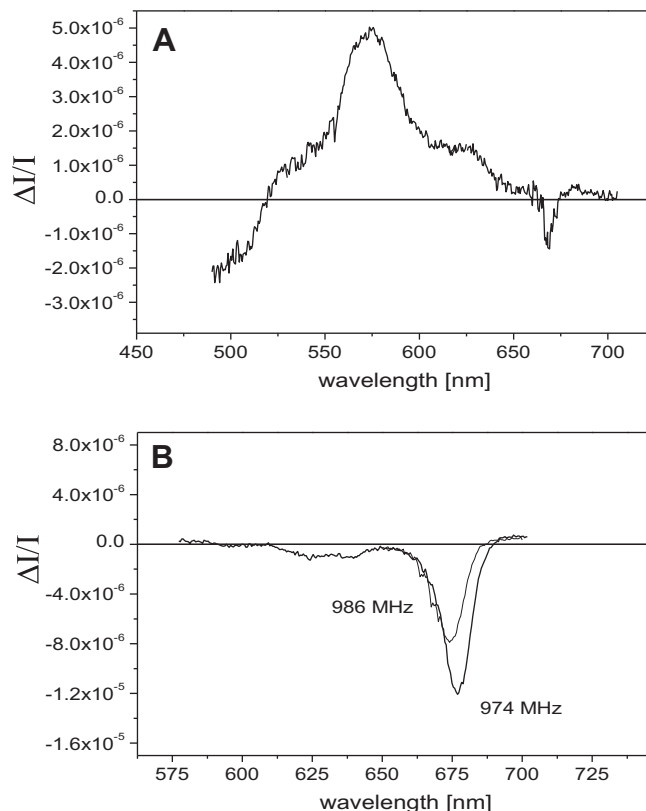


Fig. 4. T–S spectra of ^1Car and ^1Chl . T–S spectra of FCP at 1.8 K, detected at (A) 247 MHz ($2|E\rangle$ transition of the ^1Car); ampl. mod. freq., 315 Hz. (B) 986 and 974 MHz, corresponding to the $|D\rangle + |E\rangle$ transitions of selected ^1Chl states; ampl. mod. frequency, 33 Hz.

c_2 in the T–S spectra of the ^1Car states in FCP suggests that $\text{Chl } b$ is not just replaced by $\text{Chl } c$ in the FCP protein structure.

If the assignment of the two fucoxanthins located in the L1/L2 sites as quenchers of ^1Chl states is correct, the role and arrangement of the extra fucoxanthins, which are present in each FCP monomer, remains to be clarified. They are certainly not efficient in ^1Chl quenching, since a large amount of ^1Chl states is populated in the sample upon illumination, at least at low temperature. The Q_y absorption bands of the unquenched $\text{Chl } a$ molecules are seen as bleaches peaking at 672–675 nm in the associated T–S spectra, indicating that these “unprotected” Chls correspond to some “low-energy” Chls of the complex. This is the first evidence that Chls with different site energies are present in FCP. In a previous ultrafast transient absorption work on FCP [13], the absence of any spectral evolution in the $\text{Chl } a$ region following excitation in the carotenoid absorption region, suggested that the $\text{Chl } a$ molecules were spectroscopically equivalent and weakly interacting. In our measurement of the T–S spectra associated to ^1Car and ^1Chl states, we are able to distinguish between different forms of $\text{Chl } a$ having Q_y bands at 669 and 672–675 nm, respectively. The results resemble those obtained before for LHC [22]. In LHCII, $\text{Chl } a$ 611 forms a dimer with $\text{Chl } a$ 612 to yield a red-shifted absorption which is absent in FCP. $\text{Chl } a$ 611 is bound by a lipid in LHCII [4] and therefore, if a $\text{Chl } a$ molecule is bound at this site in FCP, it must have a different orientation. The altered molecular composition of the site and the change in the interactions among pigments may thus be responsible for the observed difference in the relative geometry of the Car– Chl molecules as revealed by the T–S experiments.

Compared to LHCII of plants, where Cars exhibit a 80% efficiency in singlet–singlet energy transfer, in FCP the ET from fucoxanthin

to Chl *a* is less efficient [13]. From the spectroscopic characterization of the excitation energy transfer in FCP, it has emerged that there is at least one fucoxanthin which transfers energy poorly to Chl *a*. Based on our results also the triplet–triplet ET from Chl *a* to fucoxanthin seems to be scarcely efficient.

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References

- [1] G.R. Wolfe, F.X. Cunningham, D. Durnford, B.R. Green, E. Gantt, Evidence for a common origin of chloroplasts with light-harvesting complexes of different pigmentation, *Nature* 367 (1994) 566–568.
- [2] 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- and four-helix ancestors, *Photosynth. Res.* 39 (1994) 149–162.
- [3] D.G. Durnford, R. Aebersold, B.R. Green, The fucoxanthin–chlorophyll proteins from a chromophyte alga are part of a large multigene family: Structural and evolutionary relationships to other light-harvesting antennae, *Mol. Gen. Genet.* 253 (1996) 377–386.
- [4] Z. Liu, H. Yan, K. Wang, T. Kuang, J. Zhang, L. Gui, X. An, W. Chang, Crystal structure of spinach major light-harvesting complex at 2.72 Å resolution, *Nature* 428 (2004) 287–292.
- [5] L. Premvardhan, L. Bordes, A. Beer, C. Büchel, B. Robert, Carotenoid structures and environments in trimeric and oligomeric fucoxanthin chlorophyll *a/c*₂ proteins from resonance Raman spectroscopy, *J. Phys. Chem. B* 113 (2009) 12565–12574.
- [6] L. Premvardhan, B. Robert, A. Beer, C. Büchel, Pigment organization in fucoxanthin chlorophyll *a/c*(2) proteins (FCP) based on resonance Raman spectroscopy and sequence analysis, *Biochim. Biophys. Acta. Bioenerg.* 1797 (2010) 1647–1656.
- [7] C. Büchel, Fucoxanthin–chlorophyll proteins in diatoms: 18 and 19 kDa subunits assemble into different oligomeric states, *Biochemistry* 42 (2003) 13027–13034.
- [8] E.J.G. Peterman, F.M. Dukker, R. van Grondelle, H. van Amerongen, Chlorophyll *a* and carotenoid triplet states in light-harvesting complex II of higher plants, *Biophys. J.* 69 (1995) 2670–2678.
- [9] C.C. Gradinaru, I.H.M. van Stokkum, A.A. Pascal, R. van Grondelle, H. van Amerongen, Identifying the pathways of energy transfer between carotenoids and chlorophylls in LHCII and CP29. A multicolor, femtosecond pump–probe study, *J. Phys. Chem. B* 104 (2000) 9330–9342.
- [10] H. van Amerongen, R. van Grondelle, Understanding the energy transfer function of LHCII, the major light-harvesting complex of green plants, *J. Phys. Chem. B* 105 (2001) 604–610.
- [11] B.P. Krueger, S.S. Lampoura, I.H.M. van Stokkum, E. Papagiannakis, J.M. Salverda, C.C. Gradinaru, D. Rutkauskas, R.G. Hiller, R. van Grondelle, Energy transfer in the peridinin chlorophyll-*a* protein of *Amphidinium carterae* studied by polarized transient absorption and target analysis, *Biophys. J.* 80 (2001) 2843–2855.
- [12] D. Zigmantas, R.G. Hiller, V. Sundström, T. Polívka, Carotenoid to chlorophyll energy transfer in the peridinin-chlorophyll-*a*-protein complex involves an intramolecular charge transfer state, *Proc. Nat. Acad. Sci. USA* 99 (2002) 16760–16765.
- [13] E. Papagiannakis, I.H.M. van Stokkum, H. Fey, C. Büchel, R. van Grondelle, Spectroscopic characterization of the excitation energy transfer in the fucoxanthin–chlorophyll protein of diatoms, *Photosynth. Res.* 86 (2005) 241–250.
- [14] N. Gildenhoff, S. Amarie, K. Gundermann, A. Beer, C. Büchel, J. Wachtveitl, Oligomerization and pigmentation dependent excitation energy transfer in fucoxanthin–chlorophyll proteins, *Biochim. Biophys. Acta. Bioenerg.* 1797 (2010) 543–549.
- [15] N. Gildenhoff, J. Herz, K. Gundermann, C. Büchel, J. Wachtveitl, The excitation energy transfer in the trimeric fucoxanthin–chlorophyll protein from *Cyclotella meneghiniana* analyzed by polarized transient absorption spectroscopy, *Chem. Phys.* 373 (2010) 104–109.
- [16] U. Schreiber, Detection of rapid induction kinetics with a new type of high-frequency modulated chlorophyll fluorometer, *Photosynth. Res.* 9 (1986) 261–272.
- [17] L. Dall'Osto, S. Caffarri, R. Bassi, A mechanism of nonphotochemical energy dissipation, independent from PsbS, revealed by a conformational change in the antenna protein CP26, *Plant Cell* 17 (2005) 1217–1232.
- [18] D. Carbonera, C. Gerotto, B. Posocco, G.M. Giacometti, T. Morosinotto, NPQ activation reduces chlorophyll triplet state formation in the moss *Physcomitrella patens*, *Biochim. Biophys. Acta. Bioenerg.* 2012 (1817) 1608–1615.
- [19] H.A. Frank, R.J. Cogdell, Carotenoids in photosynthesis, *Photochem. Photobiol.* 63 (1996) 257–264.
- [20] E. Salvadori, M. Di Valentin, C.W.M. Kay, A. Pedone, V. Barone, D. Carbonera, The electronic structure of the lutein triplet state in plant light-harvesting complex II, *Phys. Chem. Chem. Phys.* 14 (2012) 12238–12251.
- [21] M. Di Valentin, C. Tait, E. Salvadori, S. Ceola, H. Scheer, R.G. Hiller, D. Carbonera, Conservation of spin polarization during triplet–triplet energy transfer in reconstituted peridinin-chlorophyll–protein complexes, *J. Phys. Chem. B* 115 (2011) 13371–13380.
- [22] M. Di Valentin, E. Salvadori, G. Agostini, F. Biasibetti, S. Ceola, R.G. Hiller, G.M. Giacometti, D. Carbonera, Triplet–triplet energy transfer in the major intrinsic light-harvesting complex of *Amphidinium carterae* as revealed by ODMR and EPR spectroscopies, *Biochim. Biophys. Acta. Bioenerg.* 1797 (2010) 1759–1767.
- [23] M. Di Valentin, F. Biasibetti, S. Ceola, D. Carbonera, Identification of the sites of chlorophyll triplet quenching in relation to the structure of LHC-II from higher plants. Evidence from EPR spectroscopy, *J. Phys. Chem. B* 113 (2009) 13071–13078.
- [24] M. Di Valentin, G. Agostini, E. Salvadori, S. Ceola, G.M. Giacometti, R.G. Hiller, D. Carbonera, Triplet–triplet energy transfer in peridinin-chlorophyll *a* – protein reconstituted with Chl *a* and Chl *d* as revealed by optically detected magnetic resonance pulse EPR: comparison with the native PCP complex from *Amphidinium carterae*, *Biochim. Biophys. Acta. Bioenerg.* 1787 (2009) 168–175.
- [25] M. Di Valentin, S. Ceola, E. Salvadori, G. Agostini, G.M. Giacometti, D. Carbonera, Spectroscopic properties of the peridinins involved in chlorophyll triplet quenching in high-salt peridinin-chlorophyll *a*-protein from *Amphidinium carterae* as revealed by optically detected magnetic resonance, pulse EPR and pulse ENDOR spectroscopies, *Biochim. Biophys. Acta. Bioenerg.* 1777 (2008) 1355–1363.
- [26] R. Spezia, M. Aschi, A. Di Nola, M. Di Valentin, D. Carbonera, A. Amadei, The effect of protein conformational flexibility on the electronic properties of a chromophore, *Biophys. J.* 84 (2003) 2805–2813.
- [27] R. van der Vos, D. Carbonera, A.J. Hoff, Microwave and optical spectroscopy of carotenoid triplets in light-harvesting complex LHCII of spinach by absorbance-detected magnetic resonance, *Appl. Magn. Reson.* 2 (1991) 179–202.
- [28] D. Carbonera, G. Giacometti, U. Segre, Carotenoid interactions in peridinin chlorophyll *a* proteins from dinoflagellates: evidence for optical excitons and triplet migration, *J. Chem. Soc., Faraday Trans. 92* (1996) 989–993.
- [29] S.S. Lampoura, V. Barzda, G.M. Owen, A.J. Hoff, H. van Amerongen, Aggregation of LHCII leads to a redistribution of the triplets over the central xanthophylls in LHCII, *Biochemistry* 41 (2002) 9139–9144.
- [30] E. Hofmann, P.M. Wrench, F.P. Sharples, R.G. Hiller, W. Welte, K. Diederichs, Structural basis of light harvesting by carotenoids: peridinin chlorophyll-protein from *Amphidinium carterae*, *Science* 272 (1996) 1788–1791.
- [31] S. Santabarbara, E. Bordignon, R.C. Jennings, D. Carbonera, Chlorophyll triplet states associated with photosystem II of thylakoids, *Biochemistry* 41 (2002) 8184–8194.
- [32] D. Carbonera, G. Giacometti, G. Agostini, FDMR of carotenoid and chlorophyll triplets in light-harvesting complex LHCII of spinach, *Appl. Magn. Reson.* 3 (1992) 859–872.
- [33] R.H. Clarke, Triplet State ODMR Spectroscopy. Techniques and Applications to Biophysical Systems, Wiley-Interscience, New York, 1982.
- [34] D. Carbonera, Optically detected magnetic resonance (ODMR) of photoexcited triplet states, *Photosynth. Res.* 102 (2009) 403–414.
- [35] D. Carbonera, G. Giacometti, G. Agostini, FDMR spectroscopy of peridinin chlorophyll-*a* protein from *Amphidinium carterae*, *Spectrochimica Acta* 51A (1995) 115–123.
- [36] A. Angerhofer, F. Bornhauser, A. Gall, R.J. Cogdell, Optical and optically detected magnetic resonance investigation on purple photosynthetic bacterial antenna complex, *Chem. Phys.* 194 (1995) 259–274.
- [37] D. Carbonera, V. Burzomato, E. Bordignon, G. Giacometti, G. Agostini, P. Heathcote, H.K. Leech, Fluorescence and absorption detected magnetic resonance of membranes from the green sulfur bacterium *Chlorobium limicola*. Full assignment of detected triplet states, *J. Phys. Chem. B* 106 (2002) 7560–7568.