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The excitation energy transfer in the trimeric fucoxanthin–chlorophyll protein from *Cyclotella meneghiniana* analyzed by polarized transient absorption spectroscopy

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ABSTRACT

Polarized transient absorption spectroscopy has been applied to study the carotenoid to chlorophyll excitation energy transfer in the trimeric fucoxanthin–chlorophyll protein FCPa of the centric diatom *Cyclotella meneghiniana*. We examined the transfer pathways after excitation in the main carotenoid band ($S_0 \rightarrow S_2$ transition) with two excitation wavelengths that address either red fucoxanthins only or blue fucoxanthins and the xanthophyll cycle pigments. We were able to identify different transition dipole moments for the S_1 and the ICT state, which are assumed to be a single coupled state that transfers excitation energy to chlorophyll *a*. Furthermore we obtained different transition dipole moments for the first excited state S_1 of fucoxanthin depending on the excitation wavelength.

1. Introduction

The centric diatom *Cyclotella meneghiniana* is a eukaryotic, unicellular organism capable of oxygenic photosynthesis [1,2]. Their membrane intrinsic light harvesting systems are called fucoxanthin–chlorophyll proteins (FCPs) because of the main light harvesting carotenoid fucoxanthin. In addition to the function of light-harvesting these proteins are also responsible for the protection against a surplus of light [3,4]. Two different FCPs (FCPa and FCPb) that differ in their oligomeric state were purified from *C. meneghiniana*. FCPa is trimeric and contains mainly 18 kDa subunits with a small fraction of 19 kDa proteins, whereas FCPb solely consists of 19 kDa subunits assembled in higher oligomers [1,5]. Their pigmentation and pigment ratio differ considerably from the light harvesting complexes (LHCs) of higher plants, where the chlorophyll *a* (Chl *a*) to carotenoid ratio is ~3:1 [6,7]. In FCPs four fucoxanthin (fx) molecules are contained per four Chl *a* and one chlorophyll *c* (Chl *c*) in a protein monomer, whereas the xanthophyll cycle pigments diadinoxanthin (ddx) and diatoxanthin (dtx) are found only in substoichiometric amounts [5,8,9]. The (ddx + dtx):fx ratio (~0.07) [8] is significantly smaller than in FCPs isolated from e.g. the diatom *Phaeodactylum tricornutum*, where the ratio was found to be ~0.2 [10].

Fucoxanthin exhibits the specific spectroscopic characteristics of a carbonyl containing xanthophyll, very similar to e.g. peridinin found in dinoflagellates [11]. Coupled with their S_1 excited state carbonyl containing carotenoids show evidence of an intramolecular charge transfer (ICT) state, that is responsible for the unusual properties of these carotenoids [12,13]. The lifetime of the fx S_1/ICT state displays a pronounced solvent dependence and decreases from 60 ps in nonpolar solvents (*n*-hexane) to 30 ps in polar solvents (acetonitrile). In the latter case an additional excited state absorption (ESA) band at 635 nm appears [13,14] that is red shifted compared to the pure S_1 state in nonpolar solvents. Changes of the spectroscopic properties comparable to the solvent dependence can also be induced by the protein environment. Recent results obtained from Stark spectroscopy and ultrafast transient absorption studies [15,16] identify individual fx molecules which have different absorption maxima due to their specific position within the protein, and thus are called fx_{red} and fx_{blue} [16,17].

From DNA-sequence analysis of the diatoms *P. tricornutum* and *C. cryptica* three membrane-spanning helices were predicted and a high sequence homology to LHCII from higher plants was found especially for helix 1 and 3 [18,19]. A molecular structure for the FCPs is not yet available, but a preliminary model that is shown in Fig. 1 (bottom) has been developed by Wilhelm et al. [20] on the basis of the sequence analysis and comparison to LHC proteins

Abbreviations: C., Cyclotella; Chl, chlorophyll; DAS, decay associated spectrum; ddx, diadinoxanthin; dtx, diatoxanthin; EET, excitation energy transfer; ESA, excited state absorption; FCP, fucoxanthin–chlorophyll protein; fx, fucoxanthin; GSB, ground state bleach; HL, high light; ICT, intramolecular charge transfer; LHC, light harvesting complex; SE, stimulated emission.

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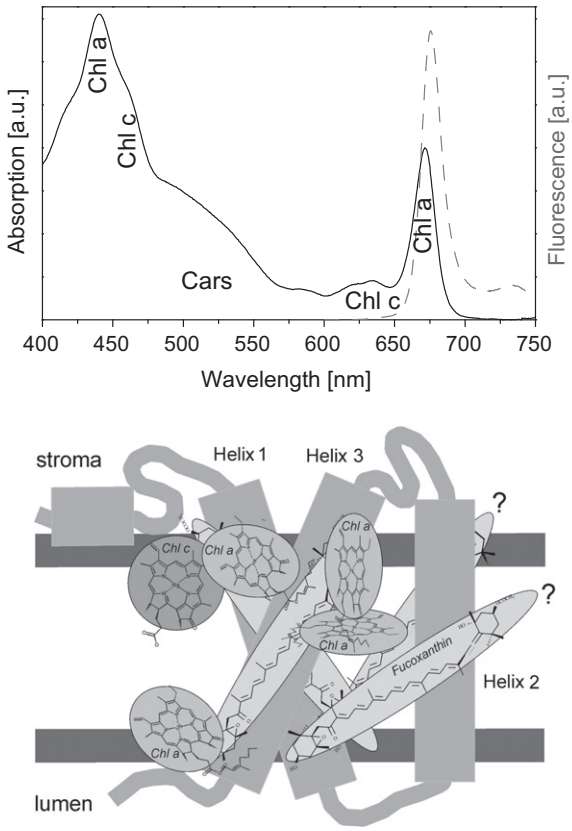

Fig. 1. Top: Steady state absorption (—) and emission (——) spectra of FCPa, the emission spectrum was measured upon excitation at 500 nm. Bottom: FCP model adopted from Wilhelm et al. [20] based on sequence homology with LHCs [19] as well as spectroscopy results [9].

[18,19] as well as first ultrafast transient absorption measurements [9]. The Chl *c* molecule is located close to one of the Chl *a* molecules, this allows a very fast and efficient energy transfer to Chl *a* upon Chl *c* excitation [9]. Two of the four fx molecules per monomer are placed parallel to helix 1 and helix 3, similar to the luteins in LHCII. The other two fx molecules were placed randomly since the region of helix 2 is not comparable to LHCII and no further structural information concerning the fx binding sites is available so far [20].

Here we examine the excitation energy transfer upon carotenoid excitation in the trimeric FCPa complex from the diatom *C. meneghiniana* by ultrafast, polarized transient absorption spectroscopy to gain further information concerning the arrangement of the different pigments within the protein and their relative position with respect to each other. Former measurements on FCPa and FCPb showed a strong excitation wavelength dependence of the kinetic traces depending on whether the blue or the red fx molecules were excited [15,21]. Hence we analyzed the FCPa sample after $\lambda_{exc} = 500$ nm and $\lambda_{exc} = 550$ nm excitation for different relative polarizations (parallel, magic angle and perpendicular) of pump and probe pulses.

2. Materials and methods

2.1. Sample preparation

The FCPa sample from the centric diatom *C. meneghiniana* was prepared as described previously [5,15]. It was isolated from cultures grown under high light conditions (145 μE m^{-2} $s^{-1}$of white light) for 10 days, which corresponded to a cell density of ~0.15 at 677 nm. The purified FCP complexes were diluted in a buffer (25 mM Tris, 2 mM KCl, 0.03% β-DDM, pH 7.4) and adjusted to an optical density of ~0.9 mm^{-1} at 671 nm.

2.2. Spectroscopic methods

Steady state absorption spectra were recorded with a UV–VIS spectrophotometer (Jasco V670), emission spectra with a luminescence spectrometer (Perkin Elmer LS 50) after excitation at 500 nm.

The transient absorption spectra using the femtosecond pump/probe technique were collected with a setup that was described earlier [22]. Briefly, the 775 nm pulses are derived from a Clark CPA 2001 femtosecond laser system operating at a repetition rate of 1 kHz. To generate excitation pulses in the visible spectral region a part of the laser output was used to operate a non-collinear parametric amplifier (NOPA). The samples were excited at 500 and 550 nm with energies of ~20 nJ/pulse and focused in the sample with a focal diameter of ~100 μm. For probing a small part of the 775 nm output was focused on a sapphire substrate, which generates single filament white light pulses (super continuum). This white light was split into two beams serving as signal and reference, both were dispersed and detected using two 42 segment diode arrays (multi-channel detection). The pump beam passed a zero-order half-wave plate that allowed easy adjustment to parallel, perpendicular, and magic angle polarization with respect to the probe beam. For all samples, the parallel ($\Delta A_{\parallel}$) and the perpendicular ($\Delta A_{\perp}$) data were used to calculate the isotropic data ($\Delta A_m = [\Delta A_{\parallel} + 2\Delta A_{\perp}]/3$) for comparison with the measured magic angle signals. During the measurements the cuvette with a pathlength of 1 mm was moved laterally to prevent multiple excitation and degradation of the sample. Before and after each time resolved measurement steady state spectra of the samples were taken to monitor their stability.

2.3. Data analysis

The data were processed and analyzed as described in [15,23]. Before the analysis the data were corrected for group velocity dispersion [24]. For the quantitative data analysis a kinetic model was used that describes the data as a sum of exponentials. A Levenberg–Marquardt algorithm fits a number of *n* global time constants (τ_i) for all wavelengths simultaneously assuming Gaussian pump and probe pulses. The *n* wavelength dependent fit amplitudes A_i (λ) represent the decay associated spectra (DAS) for each decay. From the parallel ($\Delta A_{\parallel}$) and perpendicular ($\Delta A_{\perp}$) signals we calculated the time dependent anisotropy $r(t)$ [25] that is defined as $r(t) = (\Delta A_{\parallel}(t) - \Delta A_{\perp}(t))/(\Delta A_{\parallel}(t) + 2\Delta A_{\perp}(t))$.

3. Results and discussion

3.1. Steady state spectra

In the top panel of Fig. 1 steady state absorption and emission spectra of HL-FCPa are shown. The absorption band at 440 nm is due to the Soret band of Chl *a* while the shoulder at 460 nm belongs to Chl *c*. The peaks of the Q_y bands of Chl *a* and Chl *c* are found at 670 and 635 nm, respectively. The absorption in the 480–570 nm region is caused by the $S_0 \rightarrow S_2$ transition of the different fx molecules with contributions of the xanthophyll cycle pigments ddx and dtx around 500 nm [17]. The Chl *a* fluorescence peak at 677 nm is accompanied by a vibrational band around 740 nm.