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Mapping energy transfer channels in fucoxanthin–chlorophyll protein complex

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ABSTRACT

Fucoxanthin–chlorophyll protein (FCP) is the key molecular complex performing the light-harvesting function in diatoms, which, being a major group of algae, are responsible for up to one quarter of the total primary production on Earth. These photosynthetic organisms contain an unusually large amount of the carotenoid fucoxanthin, which absorbs the light in the blue–green spectral region and transfers the captured excitation energy to the FCP-bound chlorophylls. Due to the large number of fucoxanthins, the excitation energy transfer cascades in these complexes are particularly tangled. In this work we present the two-color two-dimensional electronic spectroscopy experiments on FCP. Analysis of the data using the modified decay associated spectra permits a detailed mapping of the excitation frequency dependent energy transfer flow with a femtosecond time resolution.

1. Introduction

During the last two decades, a remarkable progress in ultrafast time-resolved spectroscopy has led to an unprecedented characterization of excitation energy transfer kinetics in a multitude of systems, including the mapping of the very first steps of the photosynthetic process occurring after the capture of a solar photon. Excitation energy cascades have been described for the light-harvesting pigment–protein complexes of photosynthetic bacteria [1–3] and plants [4,5] with femtosecond temporal resolution. On the other hand, excitation energy transfer mechanisms in light-harvesting complexes from diatoms attracted considerably less attention. The evolutionary adaptation to their aquatic (mostly marine) environment has steered these (mostly) unicellular algae to develop a photosynthetic unit with a relatively large absorption cross-section in the blue–green spectral region. On a planetary scale the environmental significance of diatoms should never be overlooked. They are estimated to be responsible for up to a quarter of primary production on Earth [6–8]. Additionally, about 16 gigatons of the organic carbon produced by marine phytoplankton per year is thought to be deposited into the ocean interior, i.e. removed from the atmosphere [6]. This is about one third of total ocean production, whereby diatoms account for 40% of the marine phytoplankton production [8], making them bigger producers than all the rainforests on Earth [7]. So diatoms are not only responsible for a lot of O_2 on this planet, but also for removal of CO_2 from the atmosphere.

In diatoms, the absorption in the blue–green spectral region is usually ensured by the carbonyl-containing carotenoid molecules, such as peridinins or fucoxanthins. The fucoxanthin–chlorophyll protein (FCP) is the main complex responsible for light-harvesting. Characterizing the electronic excitation dynamics in FCP is a particularly challenging task because of a large number of molecules and electronic levels involved. As fucoxanthin (Fx) is a carbonyl-containing carotenoid, an intramolecular charge transfer (ICT) state is present in the vicinity of the Fx S_1 state which strongly influences the excited state dynamics [9]. FCP shares structural and sequence homology with the LHCII complex from higher plants [10] and this has led to several models of pigment organization in FCP [11–13]. However, the pigment composition and function in LHCII and FCP are markedly different, as the latter contains nearly as many fucoxanthin molecules as chlorophylls *a* and *c* [11,14,12]. The best characterized fraction of FCP complexes contains no less than 8 molecules of Fx. Together with two protein-bound chlorophyll *c* (Chl *c*) molecules, they confer to the FCP its efficient absorption in the blue–green spectral region (Fig. 1).

In most pump–probe studies of dynamical processes in FCP [11,13,15,16] the 18,000–21,000 cm^{-1} region was excited to populate selectively the Fx S_2 state avoiding the Soret band of the chlorophylls

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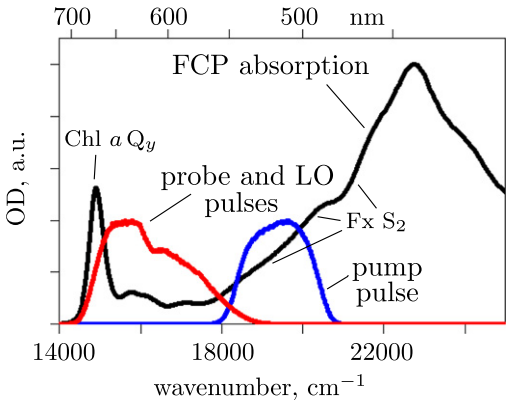

Fig. 1. FCP absorption spectrum (black line) and laser pump, probe and LO spectra used in the two-color 2D experiments (blue and red lines, respectively).

(Fig. 1). Probing at low energies allows one to follow the excitation delivery to the Q_y state of Chl *a*; while most of the excitation of the Fx S_2 state relaxes to the S_1/ICT state in less than 100 fs, a part of the S_2 population is immediately transferred to Chl *a* (within 100–200 fs). The S_1/ICT state undergoes vibrational relaxation on the timescale between 0.5 and 1 ps at the same time transferring energy to Chl *a*. The remaining excitation of the S_1/ICT state then decays to the ground state with a longer timescale (15 to 35 ps). Some spectroscopic measurements suggested the existence of multiple species of Fx, yet their distinct roles have not been explored in detail, partly due to the dependence of frequency and time resolutions in the pump–probe technique. Therefore, a comprehensive scheme of excitation energy transfer cascade remains unavailable.

Coherent two-dimensional (2D) electronic spectroscopy (ES) provides a wealth of information about the energy and charge transfer dynamics, exciton diffusion and relaxation in molecular systems [1,2,4,5,17–20,3,21,22]. In the 2D ES the temporal and spectral resolutions are not related [23], providing a huge advantage over the pump–probe techniques. Usually in the 2D ES, all the excitation pulses are of the same wavelength, or color. However, it may be hugely advantageous to tune them to different wavelengths, thus providing a way to monitor energy transfer between energetically remote excited states [24,25]. This is why in this study we employed the two-color 2D ES to disentangle the complex energy transfer cascades in FCP following the excitation of the Fx S_2 state. Application of the two-color 2D ES is very rare due to its extreme experimental difficulty [24,26–29]. This work is the first application of the two-color 2D ES to a complicated light-harvesting complex. As a result, a comprehensive scheme of energy transfer cascade in FCP following the Fx S_2 state excitation was constructed. It turns out that, although fucoxanthin absorption is stretched over a very large range of energy in these complexes, their spatial organization results in an ultrafast and efficient excitation transfer funnel from any of these molecules to the chlorophylls.

2. Materials and methods

2.1. Sample preparation

Sample preparation is described in detail elsewhere [30]. In short, cultures of the diatom *Cyclotella meneghiniana* were grown under cycles of 16 h light and 8 h dark conditions. FCP complexes were purified by sucrose density centrifugation after solubilization of the thylakoid membranes in the presence of 20 mM dodecylmaltoside. The FCP fractions corresponding to FCPa were harvested and pooled and stored until required.

2.2. 2D ES and pump–probe experiment

For the two-color 2D ES experiment, a diffractive-optics-based non-collinear four-wave mixing setup with phase-matched box geometry, heterodyne detection, and inherent phase-stabilization was used [31,32]. The light source consisted of two non-collinear optical parametric amplifiers, one home-built and one Orpheus (Light Conversion), pumped by an Yb:KGW laser (Pharos, Light Conversion). The pulse repetition rate used in the experiments was 50 kHz. The setup provided 625 nm (100 nm FWHM) 15 fs light pulses and 517 nm 22 fs light pulses (51 nm FWHM, see Fig. 1). Additional sensitivity and noise reduction was achieved by means of a double modulation lock-in detection [32]. The coherence time delay (t_1) was scanned within the −105–132 fs interval with a 1.5 fs time step by deploying movable fused-silica wedges. The population time delay t_2 was scanned using a mechanical delay stage. Measured data sets contained 40 spectra from 0 fs to 500 ps. Modified decay associated spectra fits were performed using 32 spectra up to 100 ps. Spectrometer and dispersive delay line calibration were performed using procedures described in Ref. [33]. The total energy of the sample irradiation was 1 nJ/pulse, the diameter of the focused beam at the sample was ~100 μm. To avoid local degradation of the sample at room temperature, a low volume fused silica flow cell with 0.2 mm thick windows and 0.2 mm sample chamber was used. The optical density of the sample at 680 nm was about 0.3. The frequency resolution was ~200 cm^{-1} in ω_1 and ~50 cm^{-1} in ω_3. A window function could be applied to remove the wiggles in the 2D spectra, but at a cost of ω_3 range. Thus, we chose not to use it. Pump–probe experiments were performed using essentially the same setup, just blocking two out of four laser pulses (pulses 2 and 3) and using the remaining ones as the pump (pulse 1) and probe (local oscillator) pulses.

3. Principles of two-color 2D ES

In the two-color 2D ES, the wavelengths (colors) of each laser pulse are tuned. Since the narrow-bandwidth laser pulse can trigger the resonant transitions between the electronic states, one can deduce that a wisely chosen sequence of pulse colors in the 2D ES can induce one or more specific excitation evolution pathways. This would allow extracting a specific spectral feature in 2D electronic spectrum (diagonal peak or a cross-peak) determined purely by the pulse energies and spectral bandwidths [25,34]. Here we consider the "populations-specific" two-color 2D ES experiment where the first two excitation, or pump, pulses are resonant with transitions to the high-energy state of an arbitrary system, and the frequency of the third excitation, or probe, pulse and the local oscillator (LO) pulse (which is used in the heterodyne detection [34,35,31,32]) are resonant with the transitions to the lower-energy state. A basic scheme of the experimental setup is presented in Fig. 2a; the delay between the first two pulses is denoted as t_1 (coherence time), between the second and the third as t_2 (population time), and between the third and the signal as t_3 (detection time). A 2D spectrum is obtained by performing the two-dimensional Fourier transform of the signal over delays t_1 and t_3. The Fourier frequencies ω_1 and ω_3 are denoted as the excitation and emission frequencies, respectively. The 2D spectra are then plotted against the excitation (horizontal) and the emission (vertical) axes at fixed values of the population time t_2.

In order to discuss the typical outcomes of the two-color 2D ES experiment, a simple model system of four bands of states can be considered, with an electronic ground state $|g\rangle$, a band of high-energy "blue" states $|B\rangle$, a band of low-energy "red" states $|R\rangle$ and a band of doubly-excited states $|f\rangle$. The schematic energy level diagram and absorption spectrum of this system are presented Fig. 2b along with the spectra of "blue" pump, "red" probe, and LO laser pulses. The system can be optically excited from its ground state $|g\rangle$ to states in either band $|B\rangle$ or band $|R\rangle$. The $g \leftrightarrow B$ transitions are resonant with the pump pulses, while the $g \leftrightarrow R$ and $R \leftrightarrow f$ transitions are resonant with the probe pulse. We assume that the population of the $|B\rangle$ state ($|B\rangle\langle B|$)