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Ultrafast singlet excitation transfer from carotenoids to chlorophylls via different pathways in light-harvesting complex II of higher plants

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Abstract

Sub-picosecond excitation transfer from carotenoids to chlorophyll in light-harvesting complex II was studied at 77 K using pump–probe experiments. We did not find evidence for energy transfer from the carotenoids to chlorophyll *b*. However, transfer from carotenoid to chlorophyll *a* was found to occur with a time constant of 220 ± 25 fs. It is observed that spectrally different chlorophyll *a* pools are populated upon selective excitation of different carotenoids.

1. Introduction

Light-harvesting complex II (LHCII) is the most abundant light-harvesting antenna pigment–protein complex of plants. The trimeric complex binds about half of the chlorophyll (Chl) present in the chloroplast [1,2]. Chemical (HPLC) analysis has shown that each monomer binds 5–6 Chl *b*, 7–8 Chl *a* and several carotenoid (Car) molecules: 2 luteins (Lut), 1 neoxanthin (Neo) and variable substoichiometric amounts of violaxanthin (Vio) (< 0.5) [2,3]. The Car's serve several functions in LHCII. First they protect the plant against singlet oxygen that can be formed by excitation transfer from Chl triplet states to (ground state) triplet oxygen [4]. In LHCII the Chl triplet states are quenched very efficiently by the Car's (at room temperature), thus shortening the lifetime of the Chl triplet states several orders of magnitude [5]. A second function of the Car's in LHCII is to stabilize the complex, as found from reconstitution experiments [6]. Furthermore, it was proposed that the Car's in LHCII play a role in the regulation of the energy flow from the antenna to the PSII reaction center via the xanthophyll cycle [7,8]. Finally, the Car's in LHCII harvest blue/green light that is used for photosynthesis. It has been shown [9] that the Car's in LHCII transfer their excitations very efficiently (about 100%) to Chl *a*.

An important motivation to study the Car's in LHCII is the availability of a structural model, at nearly atomic resolution (3.4 Å) [10]. The structure

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reveals a prominent position of two Car's in the center of the complex. These two Car's, the only ones that were resolved, were assigned to Lut. The structure shows short distances between the Chl's and the Lut's (for the Chl's closest to the Lut's about 4 Å), a requirement for efficient triplet and singlet energy transfer between the species [7]. The resolution of the structure was not high enough to distinguish between Chl *a* and *b*. Therefore, it was argued [10] that since triplets only are formed on Chl *a* molecules, these must be in close contact with the Lut's to explain the efficient triplet transfer. This assignment assumes that only the two resolved Lut's are involved in the triplet-quenching mechanism. This is not in agreement with recent studies of the triplets in LHCII [3,5,11], which show that at least two types of Car's are involved. The two contributions peaking at 524 and 506 nm in the 4 K *T–S* spectrum are associated with bands in the absorption spectrum at 510 and 494 nm, respectively [5]. Recently we have shown that the 510 nm Car absorption band is due to Vio [3]. Throughout this paper we will refer to the Car absorbing at 494 nm (and responsible for the 506 nm peak in the *T–S* spectrum) as the blue Car and to the one absorbing at 510 nm (*T–S* maximum at 524 nm) as the red one.

In this contribution we will deal with singlet excitation transfer from Car to Chl in LHCII. To be efficient the Car-to-Chl excitation transfer has to be ultrafast because the lifetimes of the Car excited states are very short (in the order of 200 fs for the strongly dipole allowed S_2 state and in the order of 5–25 ps (depending of the S_1 energy) for the dipole forbidden S_1 state) [12]. Car-to-Chl excitation transfer has been studied extensively for antenna complexes of purple photosynthetic bacteria (recently reviewed by Koyama et al. [12]). The transfer has been found to occur with time constants ranging from ≈ 400 fs to several ps, depending on the species, but it is still a matter of debate which transfer mechanism (coulombic or exchange), and which Car and bacteriochlorophyll states are involved [12]. Trautman et al. have performed sub-picosecond transient absorption measurements on thylakoid membrane preparations of two algae [13]. For the diatom *Phaedactylum tricornutum* (which contains fucoxanthin as a major Car) Car-to-Chl *a* transfer times (as obtained from the rise of the Chl *a* bleaching upon Car excitation) of 0.5 ± 0.1 and 2.0 ± 0.5 ps (ratio 1.7 ± 0.7) were observed. For the eustigmatophyte *Nannochloropsis sp.*, which contains Vio as a major Car (also present in LHCII), a Car-to-Chl *a* transfer time of 0.24 ± 0.04 ps was observed. Here we present data on the Car-to-Chl singlet excitation transfer in LHCII of spinach at 77 K. We show that upon selective excitation of different Car pools different Chl *a* pools are populated with a characteristic time constant of 220 ± 25 fs.

2. Materials and methods

Trimeric LHCII was prepared and purified from spinach using the method described earlier [5] based on anion-exchange chromatography and using the detergent n-dodecyl-β,D-maltoside (DM) for solubilization of the complexes. This LHCII preparation is free of minor LHC's (CP29, CP24, CP26). The pigment ratio's of this preparation were determined using HPLC [3] to be: 6 Chl *b*, 8.3 Chl *a*, 2.0 Lut, 1.0 Neo and 0.23 Vio per monomer. LHCII was diluted in a buffer containing 20 mM Hepes (pH 7.5), 0.06% (w/v) DM and 70% (v/v) glycerol. All measurements shown here were performed at 77 K in a nitrogen bath cryostat (Oxford Instruments DN1704) to obtain a higher selectivity.

Sub-picosecond pump–probe measurements were performed using pulses from the combination of an Ar ion laser pumped Ti:sapphire oscillator (Coherent MIRA 900), a Ti:sapphire regenerative amplifier (Coherent REGA 9000) and an optical parametric amplifier (Coherent OPA 9400). The repetition rate was set to 20 kHz to avoid building up of Car triplets (lifetime ≈ 12 μs [5]). The output of the OPA at 500 or 514 nm (5 nm FWHM) was attenuated to 40 μW and used as pump pulse. Unamplified white light from the OPA (< 5 μW) was used to probe absorption changes between 645 and 685 nm. The pump beam was modulated at about 1 kHz. The probe signal was measured via a monochromator (bandwidth ≈ 4 nm) with a photodiode connected to a lock-in amplifier. To avoid polarization effects, the polarization of the probe beam was at magic angle with respect to the pump beam. The instrument response of the setup had a width of 160 fs. The pump–probe optical density changes were at most 4

mOD at 680 nm. We did not observe a decrease in this signal on a time scale of tens of picoseconds indicating the absence of annihilation. Traces measured at different detection wavelengths were analyzed globally using a sequential model [14] with three components, characterized by their lifetimes and species associated spectra. The fits for the two datasets with excitation at 500 and 514 nm shown here are the result of a simultaneous fit of both datasets in which the decay times were kept the same (but the spectra different) for both datasets. Independent fits for both datasets yield the same results within the given error margins.

3. Results

In Fig. 1 the 77 K absorption spectrum of LHCII is shown. The excitation and detection wavelengths of the pump–probe experiments are indicated. Reconstruction of the LHCII absorption spectrum in the Car S_2 region, based on the pigment stoichiometry, *T–S* and fluorescence excitation measurements [15] have shown that excitation at 500 and 514 nm is most selective for excitation of the blue and red Car, respectively (see introduction). The reconstruction also shows that a considerable amount of Chl *b* is excited at these wavelengths (about 25%). In Fig. 2 we show several traces for both excitation wavelengths. To be able to judge the quality of the global fits, the fitted traces are also indicated. In Fig. 3 the results of the fits are shown: the kinetic model fitted to both datasets and the species associated spectra.

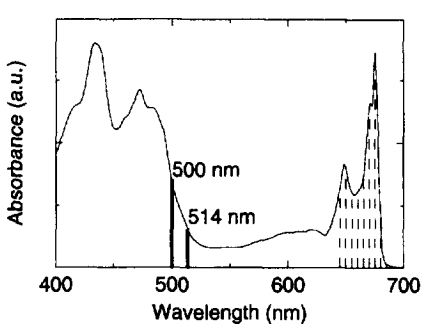

Fig. 1. Absorption spectrum of LHCII at 77 K, spectral bandwidth 0.5 nm. Indicated are the wavelengths of excitation (thick vertical lines) and detection (black, dashed).

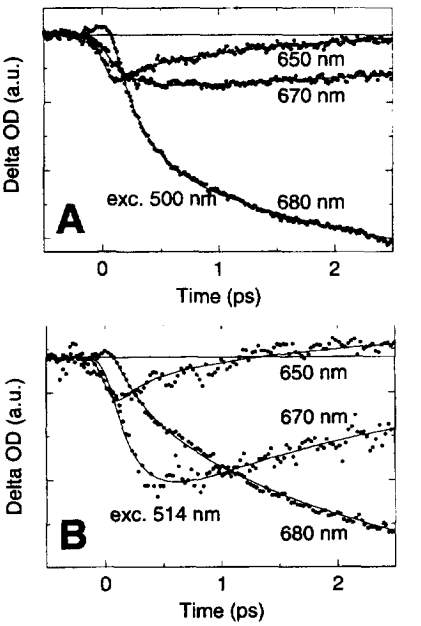

Fig. 2. Transient absorption traces measured on LHCII at 77 K excited at (A) 500 and (B) 514 nm. The detection wavelengths are indicated. Shown in the figures are the datapoints (dots) and the fits (solid line).

The first spectrum, which grows instantaneously with the pump pulse, shows for both excitation wavelengths bleaching/stimulated emission (SE) of Chl *b* around 650 nm, arising from direct excitation of Chl *b*. This bleaching corresponds to 25% (for 500 nm excitation) and 30% (for 514 nm excitation) of the final Chl *a* bleaching (taking into account the smaller extinction coefficient of Chl *b* ($\approx 60\%$) [16]). This is similar to what we expected based on the reconstructed absorption spectrum [15]. Also observable in this first spectrum are some small, positive features at 680 and 665 nm. It is tempting to assign these to excited state absorption (ESA) of the Car's.

This instantaneous spectrum is replaced in 220 ± 25 fs by one that shows considerable bleaching/SE in the Chl *a* region (670–680 nm). Interesting to note is that the maximum of the Chl *a* bleaching/SE is at different wavelengths for the two excitation wavelengths: at 675 nm for 500 nm excitation and at 670 nm for 514 nm excitation. These spectra show slightly less Chl *b* bleaching/SE than the instanta-

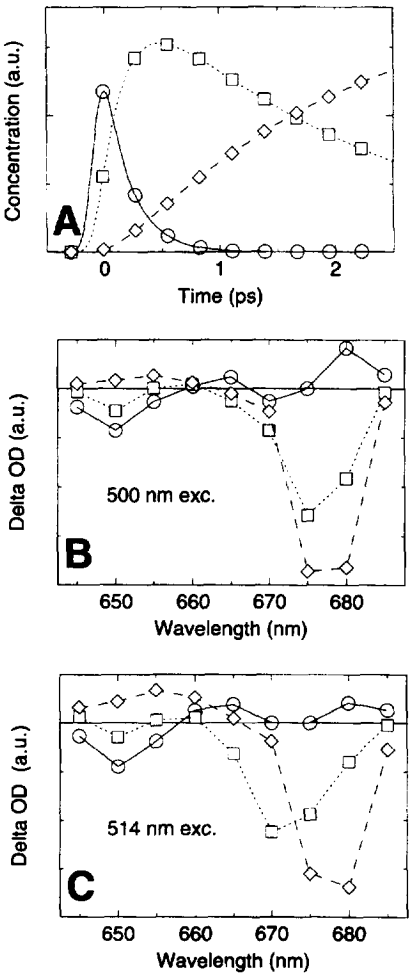

Fig. 3. Results of the global analysis of the pump–probe measurements on LHCII at 77 K. (A) Kinetic model fitted to both data sets; (B) and (C), species associated spectra of the fit of the (B) 500 and (C) 514 nm excited datasets. In all three pictures similar symbols and linetypes are used for the same components: the first component (solid line, circles) decays in 220 ± 25 fs, the second component (dotted, squares), which decays in $2.1 + 0.25$ ps to the third component (dashed, diamonds).

neous ones, which is mainly caused by a cancellation of the signal by Chl *a* excited state absorption. To some extent it might also be due to fast Chl *b*-to-*a* transfer. In ultrafast fluorescence and transient absorption experiments of LHCII in which Chl *b* was excited in the Q_y band, transfer times in this order of magnitude have been observed [17–19]. We estimate that this decrease in the Chl *b* signal can account for about 10% of the amplitude of the Chl *a* signal. We assign this second component mainly to the rise of Chl *a* bleaching/SE upon transfer from the Car's. Clearly the Car that is excited at 514 nm (Vio) transfers its excitation to a relatively blue absorbing (670 nm) Chl *a* pool and the Car's excited at 500 nm (Lut and possibly Neo) to Chl *a* that absorbs more to the red (675 nm). This coupling of a red Car with a Chl *a* at 670 nm and a blue Car with a Chl *a* near 675 nm has been observed before in ADMR experiments by van der Vos et al. [11]. They observed changes in absorption at 676 nm upon selective microwave excitation of the blue Car triplet and at 670 nm upon excitation of the red Car triplet. Furthermore, we recently observed that selective excitation at 4 K of the red Chl *a*'s (680 nm) leads to increased population of blue Car triplets and excitation more to the blue (670 nm) to increased population of red Car triplets [15].

In 2.1 ± 0.25 ps the second spectrum is replaced by the final one, which is very similar for both excitation wavelengths: bleaching/SE of Chl *a* at 675–680 nm and some ESA of Chl *a* around 655 nm. Several processes contribute to the formation of this spectrum: Chl *b*-to-*a* transfer, Chl *a* equilibration and possibly also vibrational cooling. The 2.1 ps time constant reflects all the processes mentioned above. It is not due to slow ($\approx$ ps) Car-to-Chl excitation-transfer, since the total Chl bleaching, corrected for the different extinction coefficients of Chl *a* and *b*, is roughly the same as for the spectrum of the second component (not more than 15% difference). For the Chl *b*-to-*a* transfer fast times have been observed (200–600 fs) but also slower ones (3–10 ps) [17–19]. Also Chl *a* equilibration takes place over a relatively large time range: fast (< 300 fs), intermediate (≈ 1–2 ps) and slow (≈ 10–20 ps) components have been identified [18,19]. The limited time window of our measurements (5 ps) does not allow discrimination between all these processes in our data.

4. Discussion

We have shown that Car-to-Chl *a* transfer takes place in 220 ± 25 fs and that upon excitation of different Car's spectrally different Chl *a* molecules

are populated. It is interesting to note that this transfer time is similar to the 240 ± 40 fs found for Vio-to-Chl *a* transfer in the eustigmatophyte *Nannochloropsis sp.* [13]. In the present study the situation is more complicated since also Chl *b* is present. The first, instantaneous component of the global analysis of our data already shows Chl *b* bleaching/SE, which we interpret as direct excitation of Chl *b*, leading to about 25–30% of the final Chl *a* bleaching/SE. This is in line with our recent reconstruction of the LHCII absorption spectrum in the 400–550 nm region, which indicates that both at 500 and 514 nm about 25% of the absorption is due to Chl *b* [15]. However, we cannot exclude that part of the directly bleached Chl *b* is due to Car-to-Chl *b* transfer. If this were the case this Car-to-Chl *b* excitation transfer should be very fast, too fast to detect with our setup. Introduction of an extra component in the global analysis did not improve the quality of the fit and showed no evidence for Car-to-Chl *b* transfer.

Also at later times no increase in the Chl *b* bleaching/SE is observed. Only if the Chl *b*-to-*a* transfer would be much faster than the Car-to-Chl *b* transfer, we would not observe such an increase. Since the fastest Chl *b*-to-*a* transfer has been estimated to take place in about 200 fs [18,19] (due to about 40% of the Chl *b*'s in LHCII [19]), the overall Car-to-Chl *a* transfer via this pathway would have to take much longer than 200 fs, which is not in agreement with the observed rise time of 220 fs for the Chl *a* bleaching/SE. We can therefore exclude that such a pathway is the major one. However, given the experimental uncertainty of our data it is possible that parallel to the direct Car-to-Chl *a* transfer a minor fraction of the transfer has Chl *b* as an intermediate. In summary, we observe a 220 ± 25 fs rise time of the Chl *a* bleaching/SE upon Car excitation which is mainly due to direct transfer from Car to Chl *a*.

The finding that the Car's transfer their excitations mainly to Chl *a* is in agreement with the results of *T–S* measurements [5,11], which show evidence for interactions between the Car's and Chl *a* but not between the Car's and Chl *b*. The *T–S* measurements [11,15] showed that these interactions are specific: the red absorbing (510 nm) Car seems to be connected to relatively blue absorbing Chl *a* and the blue absorbing (494 nm) Car to relatively red absorbing Chl *a*. In agreement with these measurements we observe that excitation of the red Car (at 514 nm) leads to direct population of relatively blue absorbing Chl *a* (670 nm) and excitation of the blue Car (at 500 nm) to direct population of relatively red absorbing Chl *a* (675 nm).

Again [3,5,11] we have shown that apart from the Lut's, Vio also plays an important functional role in LHCII. The fast excitation transfer from Vio to Chl *a* requires close proximity of the two species. This finding questions the validity of the assumption made for the assignment of the Chl *a*'s in LHCII (close proximity of all Chl *a*'s to the two central Lut's) [10]. However, it does not invalidate the Chl assignment since the structural model is missing several Chl and Car molecules.

In principle transfer could take place from the Car S_2 and S_1 state to Chl *a* Q_y, Q_x and vibronic states via coulombic and/or exchange mechanisms. Based on our data we cannot distinguish between these mechanisms. We only want to mention here that if the time constant for internal conversion of the Car S_2 to S_1 state is on the order of 200 fs, like for β-carotene [12], a Car-to-Chl *a* transfer time of 220 fs and a transfer efficiency of nearly 100% [9,15] implies that the S_2 state cannot be the only state from which transfer to Chl *a* takes place.

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