Ultrafast excitation dynamics of low energy pigments in reconstituted peripheral light-harvesting complexes of photosystem I

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Abstract Ultrafast dynamics of a reconstituted Lhca4 subunit from the peripheral LHCI-730 antenna of photosystem I of higher plants were probed by femtosecond absorption spectroscopy at 77 K. Intramonomeric energy transfer from chlorophyll (Chl) *b* to Chl *a* and energy equilibration between Chl *a* molecules observed on the subpicosecond time scale are largely similar to subpicosecond energy equilibration processes within LHCII monomers. However, a 5 ps equilibration process in Lhca4 involves unique low energy Chls in LHCI absorbing at 705 nm. These pigments localize the excitation both in the Lhca4 subunit and in LHCI-730 heterodimers. An additional 30–50 ps equilibration process involving red pigments of Lhca4 in the heterodimer, observed by transient absorption and picosecond fluorescence spectroscopy, was ascribed to intersubunit energy transfer.

Key words: Antenna; Excitation energy transfer; Light-harvesting complex; LHCI-730 heterodimer; Time-resolved spectroscopy; Photosystem I

1. Introduction

The photosystem I (PSI) holocomplex in thylakoids of chloroplasts in higher plants consists of a PSI core antenna and energy-supplying peripheral light-harvesting complexes (LHCI-680 and LHCI-730). A fingerprint of the PSI holocomplex is its low temperature fluorescence emission at 735–740 nm in vivo [1,2] and in vitro [3,4], more than 80% of which originates from LHCI-730 [5]. Recent reconstitution of the LHCI-730 [6] proved that the complex assembles as a heterodimer of Lhca4 and Lhca1 subunits and resembles biochemically and spectroscopically native LHCI-730 complexes. The red-shifted fluorescence of the LHCI complexes was localized to the Lhca4 subunit of the LHCI-730 subpopulation in vitro [6,7].

Similar protein sequences of pigment-binding regions and predicted folding patterns in all light-harvesting complexes of higher plants indicate that basic functional elements are conserved in all light-harvesting complexes of higher plants [8]. A current 3.4 Å structural model of Lhcb1 monomer of the LHCII trimer [9] includes three membrane spanning helices binding 12 chlorophyll (Chl) molecules with Chl *a* in close contact with Chl *b* for rapid energy transfer and two crossed lutein molecules possibly quenching Chl *a* triplet states. This structure is broadly consistent with time-resolved studies of LHCII complexes that report intrasubunit Chl *b* to Chl *a* energy transfer and spectral equilibration between Chl *a* spectral forms towards a longer wavelength state at 679 nm occurring on subpicosecond and picosecond time scales with time constants falling into two groups, 0.2–0.7 ps and 2–9 ps [10–12]. Energy transfer between monomers in the LHCII trimer probably occurs on the 10–20 ps time scale [10,13].

An earlier picosecond fluorescence study of detergent-isolated spinach bulk LHCI complexes at 77 K and excitation at 650 nm revealed two energy transfer processes with lifetimes of 30 ps and 200 ps, populating low energy Chl *a* species absorbing at 708 nm and emitting at 735 nm [14]. For native bulk LHCI, a 200–400 fs depolarization was reported [15] indicating the fast hopping time between individual pigments. However, in native LHCI-730 complexes, no fast depolarization phase except a 15 ps process was observed at room temperature and excitation at 670 nm [15]. The 15 ps phase was assigned to a transfer of excitation energy to the pigments giving rise to far-red emission component F735. Picosecond fluorescence spectroscopy of reconstituted Lhca1 and Lhca4 monomers at room temperature [16] did not find any decaying processes in either monomer faster than 200 ps. However, in LHCI-730 heterodimers, which bind additional Chl *b* and characterized by changed pigment–pigment and pigment–protein interactions [6], an additional 30–50 ps energy transfer component was found and ascribed to intersubunit energy redistribution, from Lhca1 to Lhca4 [16].

This communication is the first on 77 K ultrafast dynamics of reconstituted subunits of LHCI-730. Subpicosecond energy equilibration ($\sim$500 fs) between Chl *b* and Chl *a* spectral forms shows reconstruction of pigment–protein and pigment–pigment interactions characteristic for functional LHCI. In contrast to LHCII complexes, intramonomeric equilibration among Chl *a* spectral forms includes Lhca4-specific red pigments absorbing at 705 nm.

2. Materials and methods

Reconstituted (r)-Lhca1, Lhca4 and LHCI-730 polypeptides from tomato were prepared from overexpressed apoproteins, Chl *b*, Chl *a* and carotenoids using protocols described earlier [6]. Chl *a*/*b* ratios of r-Lhca1, r-Lhca4 and r-LHCI-730 were 3.2, 2.4 and 2.6, respectively.

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For time-resolved absorption spectroscopy, the samples were resuspended in 20 mM Tricine–NaOH, pH 7.8, containing 0.04% β-dodecylmaltoside, 66% glycerol and frozen to 77 K using a liquid nitrogen optical cryostat Optistat (Oxford). Transient absorption spectra of reconstituted LHCI polypeptides at 77 K were measured using the femtosecond spectrometer described earlier [17]. The samples were excited at 640, 645 and 650 nm with 200 fs laser pulses (fwhm = 6 nm). Transient absorption spectra were measured on 4, 50 and 200 ps time scales in the 600–750 nm spectral region. The level of spectral noise was about 0.003 ΔA. The kinetics were analyzed globally based on a model of exponential decay of Chl excited states. The wavelength plots of initial amplitudes of the exponential components represent decay-associated spectra (DAS) with negative and positive amplitudes meaning Chl excitation decay and rise (appearance of new spectral bands), respectively.

Picosecond fluorescence of r-LHCI-730 polypeptides was measured by time-correlated single-photon counting (SPC) using excitation at 610 nm described earlier [16].

3. Results and discussion

3.1. r-Lhca4 monomer binds red pigments

Fig. 1 compares transient absorption spectra of reconstituted Lhca1, Lhca4 and LHCI-730 measured at 50 ps time delay and excitation of Chl *a* at 645 nm. In contrast to r-Lhca1, excitation of pigments in r-Lhca4 and r-LHCI-730 complex localizes on a pool of Chl *a* absorbing at 705 nm. No spectral pools of Chls absorbing around 705–710 nm were populated either on shorter or longer time scales in r-Lhca1 (data not shown). This further proves that low energy pigments in the LHCI-730 heterodimer originate in Lhca4 in agreement with earlier reports [6,7]. An extension of the broad ΔA band of red pigments in spectra of r-Lhca4 and LHCI-730 further to the red is due to a stimulated emission reflecting a build up of the fluorescence at 730 nm [6].

3.2. Intrasubunit energy equilibration in r-Lhca4

Pigment–pigment and pigment–protein interactions in the reconstituted Lhca4 monomer were probed at 77 K by measuring of transient absorption spectra upon excitation of Chl *b* at 640 nm (Fig. 2). Several energy transfer processes on the subpicosecond time scale are clearly resolvable. Broadening of the initial ΔA band at 640 nm within 1 ps indicates a subpicosecond energy relaxation within spectrally distinct pools of

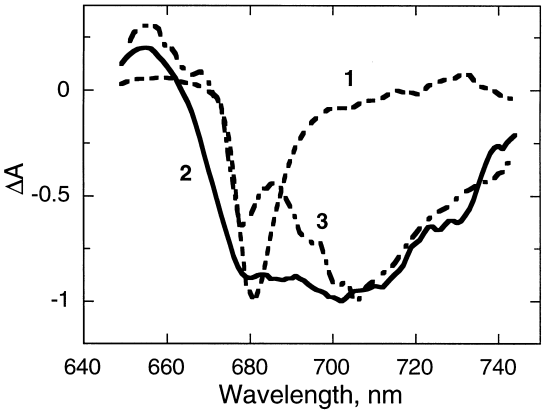

Fig. 1. 77 K transient absorption spectra of reconstituted Lhca1 (1) and Lhca4 (2) monomers, and LHCI-730 heterodimers (3) measured at 50 ps time delay with excitation at 645 nm. Spectra are normalized at maximum ΔA.

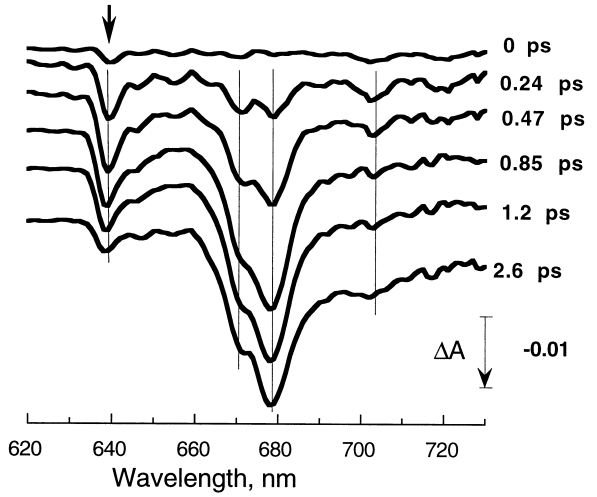

Fig. 2. 77 K ultrafast excitation dynamics induced by excitation of Chl *b* at 640 nm in r-Lhca4 monomer. Time zero is defined at ΔA comparable with an experimental noise. Transient absorption spectra are presented at representative pump–probe delays. The wavelength axis in spectra is offset for clarity. Vertical lines at 640, 670, 679 and 703 nm are added for comparison of spectra.

Chl *b*. A prompt photobleaching of Chl *a* at 670 and 679 nm accompanying the initial ΔA band at 640 nm is due to excitation of pigments via broad vibronic bands of Chl *a*. Weak absorption changes in the initial transient spectra around 705 nm are within the noise level. A 500 fs decay at ΔA_{640} (not shown) and a decrease of the $\Delta A_{640}/\Delta A_{679}$ ratio from 1 to 0.08 indicates a dominating ~500 fs process of energy transfer from Chl *b* to Chl *a* absorbing at 670 nm and 679 nm. Furthermore, a change of the $\Delta A_{670}/\Delta A_{679}$ ratio suggests a subpicosecond redistribution of excitation between two pools of Chl *a*. A largely similar pattern of spectral changes was reported for Lhcb1 monomer of the LHCII trimer [10]. This suggests general similarities in the structure and pigment organization of r-Lhca4 and Lhcb1 monomers. However, this similarity should not be overemphasized because in r-Lhca4 subunits on the picosecond time scale, the spectral equilibration involves a unique pool of low energy Chls with broad absorption around 705 nm (Fig. 2). Details of this energy transfer process, that probably starts on subpicosecond time scale, were probed on a longer time scale with excitation at 650 nm (Fig. 3). Global analysis of kinetics on a 50 ps time scale in the 600–750 nm region reveals two energy transfer components with lifetimes of 0.6 ps and 4.7 ps and a non-decaying (ND) component (Fig. 3A). A 0.6 ps DAS reflects a subpicosecond energy transfer from Chl *b* absorbing at 650 nm (see fast decay in Fig. 3B) to Chl *a* at 679 nm. This intrasubunit subpicosecond phase with lifetimes varying from 200 fs to 700 fs was also observed in LHCII trimers and monomers [11,12]. A 4.7 ps DAS represents an energy equilibration process between Chl *a* spectral forms in the Qy band and the red pigments. The decay and the rise in kinetics at 679 and 705 nm in Fig. 3B are dominated by this process. Similar lifetimes (~3 ps) for Chl *a* energy equilibration were reported for LHCII monomers [12], however, the red most Chl *a* spectral form in LHCII is centered at 679 nm [10,12,13]. A broad ΔA around 700–740 nm in the ND spectrum in Fig. 3A reflects the fact that excitation is localized by red pigments. Uncoupled Chl *b* and Chl *a* at 650 and 680 nm,

respectively, contributing to the ND spectrum are probably due to partial refolding of r-Lhca4.

3.3. Energy redistribution in r-LHCI-730 heterodimer

For LHCII polypeptides, the 2–9 ps Chl *a* equilibration process was ascribed to intrasubunit energy redistribution [10,12,13] while the intersubunit energy transfer in LHCII trimers was thought to be in the range of tens of picoseconds [13]. Surprisingly, no energy transfer process longer than ~5 ps excitation equilibration between Chl *a* spectral forms was observed in r-Lhca4 subunit either on the 50 ps time scale (Fig. 3) or on the 200 ps time scale (data not shown). However, in the r-LHCI-730 heterodimer, we detected a significantly slower equilibration process between Chl *a* at 679 nm and the red pigments (Fig. 4). A 200 ps transient spectrum in Fig. 4A is dominated by a broad ΔA band centered at 705 nm, indicating localization of the excitation on the Lhca4 subunit of the heterodimer. Global analysis of the kinetics returns two exponential components with lifetimes of 4 ps and 33 ps and a long-lived component ND within 200 ps (Fig. 4B). The 4 ps DAS reflects a sum of intramonomeric Chl *a* excitation equilibration phases within Lhca4 subunit (see 4.7 ps DAS in Fig. 3A) and Lhca1 subunit (data not shown). The 33 ps energy transfer process (see the 33 ps DAS in Fig. 4B) populates red spectral forms of Chl *a* with absorption at 705 nm. Excitation at 680 nm induces a similar process with a lifetime of 47 ps (data not shown). An independent proof for the presence of this energy transfer process in r-LHCI-730 was obtained by SPC at 77 K (Fig. 4C). Negative amplitudes in the 46 ps DAS correspond to a fluorescence rise at 730 nm resulting from the population of low energy Chl *a* species (see the 33 ps DAS in Fig. 4B). The 150 ps fluorescence DAS is tentatively ascribed to a rise of fluorescence band of aggregates after energy redistribution (work in progress). The 3.2 ns and 7 ns DAS reflect the phases of overall excitation decay of LHCI-730. These fluorescing states result from the excitation localization process presented by the ND component in the global analysis data of Fig. 4B.

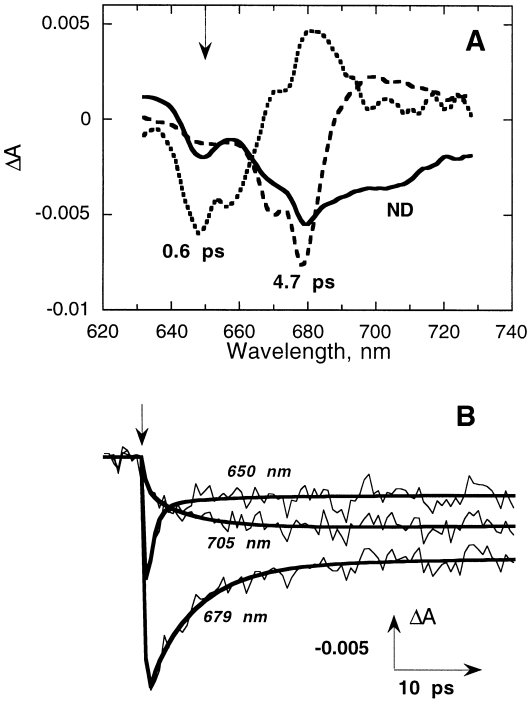

Fig. 3. DAS (A) obtained in a global analysis of transient kinetics measured in r-Lhca4 at 77 K on 50 ps time scale with excitation at 650 nm. The spectral features of the 0.6 ps DAS at 660, 675 and 700–720 nm are within the noise level. (B) Representative kinetics at 650, 679 and 705 nm are fit to the three exponential components (thick solid lines) shown in A.

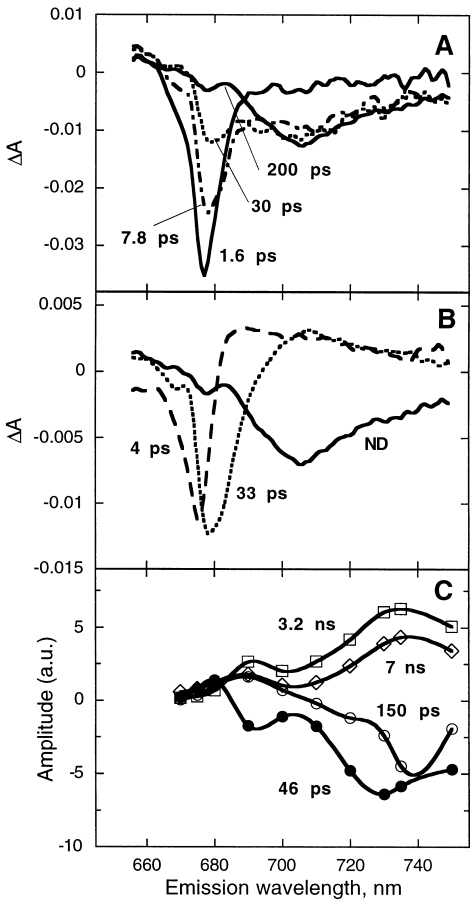

Fig. 4. (A) Transient absorption spectra of r-LHCI-730 at 77 K measured on 200 ps time scale with excitation at 645 nm. Noise level is less than 0.003 ΔA. Spectra are presented at representative pump–probe delays. (B) DAS obtained by global analysis of transient kinetics on the 200 ps time scale. (C) Fluorescence DAS obtained by global analysis of picosecond fluorescence kinetics measured by SPC in r-LHCI-730 at 77 K with excitation at 610 nm (fwhm of IRF is 30 ps).

Energy transfer processes of the same order of magnitude were observed earlier with bulk LHCI at 77 K [14] and native LHCI-730 at room temperature [15]. However, recent analysis of picosecond fluorescence in r-Lhca1 and r-Lhca4 subunits and reconstituted and native LHCI-730 complexes showed [16] that at room temperatures, this 30–50 ps energy transfer process could be ascribed to intersubunit energy redistribution between Lhca1 and Lhca4. Variations in the lifetimes possibly reflect different experimental approaches and sample heterogeneity.

In conclusion, the observed low temperature subpicosecond energy equilibration between Chl *b* and Chl *a* spectral forms in a reconstituted Lhca4 subunit indicates reconstruction of pigment–protein and pigment–pigment interactions typical for functional Chl *a*/*b*-binding light-harvesting polypeptides of

higher plants. The 5 ps intramonomeric equilibration among Chl *a* spectral forms includes Lhca4-specific low energy Chls that localize the excitation in the LHCI-730 heterodimer.

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