Excitation Energy Transfer in the Lhca1 Subunit of LHC I-730 Peripheral Antenna of Photosystem I

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Femtosecond transient absorption spectroscopy at 77 K was used to study excitation energy transfer processes in the reconstituted (r-)Lhca1 subunit of the peripheral light-harvesting antenna complex LHCI-730 of Photosystem I from higher plants. Under selective excitation of chlorophyll (Chl) *b* at 645 nm with 200 fs laser pulses, two energy transfer processes from Chl *b* to spectral forms of Chl *a* with lifetimes of 500 fs and 2.8 ps are induced. The subpicosecond phase reflects the energy transfer between closely located Chl *b* and Chl *a* molecules. Excitation at 670 nm induces a similar ultrafast process of energy transfer between neighboring Chl *a* molecules with a lifetime of 300 fs. The kinetic processes with the lifetime of 3−4 ps indicate an excitation equilibration among pools of Chl *b* and Chl *a* in the r-Lhca1, which involves the longest-wavelength spectral form of Chl *a* at 684 nm. This is in contrast to the Lhca4 subunit of the LHCI-730 heterodimer, which binds the longest-wavelength (red) pigments absorbing at 700−705 nm and responsible for the red fluorescence at 735 nm in LHCI. The spectral pattern of transient absorption changes in the r-Lhca1 is similar to that of the minor light-harvesting complex CP29 of PSII with which the Lhca1 shares significant similarity in protein sequences. A tentative assignment of the observed Chl spectral forms to the pigments in the Lhca1 subunit is presented based on the derivative absorption spectroscopy, transient absorption spectra, and the suggestion that the pigment−protein interactions dominate the spectral heterogeneity of the Q_y transitions of Chl *a* and *b*.

Introduction

Photosystem I (PS I) complex in chloroplasts of higher plants and green algae consists of the energy converting reaction center and a peripheral energy-supplying light-harvesting complex (LHCI). The unique feature of the PS I holocomplex (PSI core + LHCI) is its red fluorescence emitting at 730 nm. More than 80% of the red fluorescence in the PSI holocomplex originates from LHC I-730.[1]

The three-dimensional structure of the LHC I polypeptides is unknown, however, they are biochemically well characterized. The peripheral antenna of PSI consists of four related polypeptides, Lhca1, Lhca2, Lhca3, Lhca4, forming dimeric complexes around the PSI core.[2,3] Two subpopulations of the dimers, LHCI-680 and LHCI-730, are spectroscopically distinct with low-temperature emission dominating at 680 and 730 nm, respectively. On the basis of similar protein sequences and basic folding properties of all chlorophyll *a/b* binding polypeptides in higher plants,[4,5] the current 3.4 Å structure of the LHCII might serve as a working model for the LHCI polypeptides.[6] In this model, three transmembrane helices bind 12 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. It was suggested that two central lutein molecules and the ion pairs between helices 1 and 3 stabilize the structure. In contrast to LHCII, the second carotenoid-binding site may be occupied by substoichiometrically bound violoxanthin and *β*-carotene in LHCI polypeptides[7,8] and neoxanthin and violoxanthin in minor Chl *a/b*-binding proteins of Photosystem II.[9,10] Bassi et al.[10] for CP29 polypeptide (Lhcb4) reported that the complex is stable with only one lutein molecule.

The structure of LHCII is broadly consistent with time-resolved studies of LHCII that report intrasubunit Chl *b* to Chl *a* energy transfer and spectral equilibration between Chl *a* spectral forms toward 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.[11−13] Energy transfer between monomers in the LHCII trimer probably occurs on the 10−20 ps time scale.[13]

Excitation energy transfer processes in the LHCI-730 are poorly understood. For native bulk LHC I, the hopping time for individual pigments was suggested to lie in the range of 200−400 fs.[14] Recent analysis of reconstituted polypeptides of LHCI-730[7] showed that the complex assembles as a heterodimer of two polypeptides, Lhca1 and Lhca4. Steady-state fluorescence excitation spectra of the LHCI-730 complex indicate an effective energy transfer from carotenoids and Chl *b* to the pigments responsible for the red emission that dominates the steady-state spectra of LHCI-730 both at room temperature and 77 K.[7,15] Recent picosecond absorption and fluorescence spectroscopy study of reconstituted LHCI-730 and its monomeric constituents[15,16] concluded that Lhca1 and Lhca4 are significantly

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different from each other in terms of the sites of energy localization. Heterodimerization was suggested to induce a 30 ps intersubunit excitation energy flow from Lhca1 toward Lhca4. Recently, 77 K transient absorption difference spectra of r-Lhca4 upon excitation of Chl *b* revealed the presence of several ultrafast energy transfer processes with yet-unresolved lifetimes.[16] Two major energy transfer processes include a 400−600 fs energy transfer between spectral forms of Chl *b* and Chl *a* followed by 3−5 ps energy equilibration between Chl *a* molecules, including those involved in the red pigment's transition at 705 nm, which gives rise to the red emission of the LHCI-730 heterodimer.

The structural and functional role of the Lhca1 subunit in the LHC I is unknown. In contrast to the Lhca4 subunit with its specific molecular arrangement of the pigments giving rise to the red emission,[17,18] the spectral properties of the Lhca1 part of the LHCI-730 heterodimer are thought to be similar to those of LHCII[16] with the longest-wavelength absorbing pool of Chls localizing the excitation at 679−680 nm. The protein sequence of the Lhca1 polypeptide has the closest resemblance with the Lhcb4 (CP29) polypeptide (49% identity) with similar Chl *a/b* ratio.[19] In LHCII trimers, spectral equilibration between Chl *a* spectral forms toward the longest wavelength state at 679 nm occurs on subpicosecond and picosecond time scales with time constants of 2 ps, 10−20, and 0.4 ps at 662, 670, and 672 nm, respectively.[20] These dynamics are typical for intramonomeric excitation energy equilibration, because studies of LHCII monomers[11] and monomeric CP29 proteins[12,21] have drawn similar conclusions.

The function of the Lhca1 subunit might be the light harvesting and transfer of energy to the Lhca4 subunit. This is consistent with observation of ∼30 ps energy redistribution process in the LHCI-730 antenna assigned to the intersubunit Lhca1-Lhca4 energy transfer process.[15,16] So far, the ultrafast energy equilibration processes in the Lhca1 subunit are unknown.

This communication presents the main kinetic features of the ultrafast excitation dynamics in the Lhca1 subunit of the LHC I-730 heterodimer in the peripheral antenna of Photosystem I. In contrast to the Lhca4 subunit of the LHC I-730 heterodimer, which possesses a pool of red pigments absorbing at 705 nm, the longest-wavelength absorbing pool of Chls in the Lhca1 subunit localizes the excitation at 684 nm. Intramonomeric energy equilibration processes between pools of Chl *a* and Chl *b* were found to be as fast as in the r-Lhca4 and minor light-harvesting complexes of PS II.

Experimental Section

Reconstituted Lhca1 polypeptides from tomato were prepared from overexpressed apoproteins and total pigment extract using protocols described earlier.[7,17]

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.[16] The samples were excited at 645 and 670 nm with 200 fs laser pulses (fwhm = 6 nm). Mutual orientation of the polarization vectors for pump and probe beams was set at magic angle (54.7°). Transient absorption spectra were measured on 5 and 200 ps time scales in the 600−750 nm spectral region. The kinetic data were corrected for dispersion and were analyzed globally based on a model of exponential decay of Chl excited states. The analysis included convolution with the instrument response function of the experimental setup (fwhm = 400 fs). 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. All experiments have been performed in annihilation-free regime. On the basis of estimation of energy density per pulse,[22] a probability of excitation of one Lhca1 complex was less than 0.3 photons per pulse.

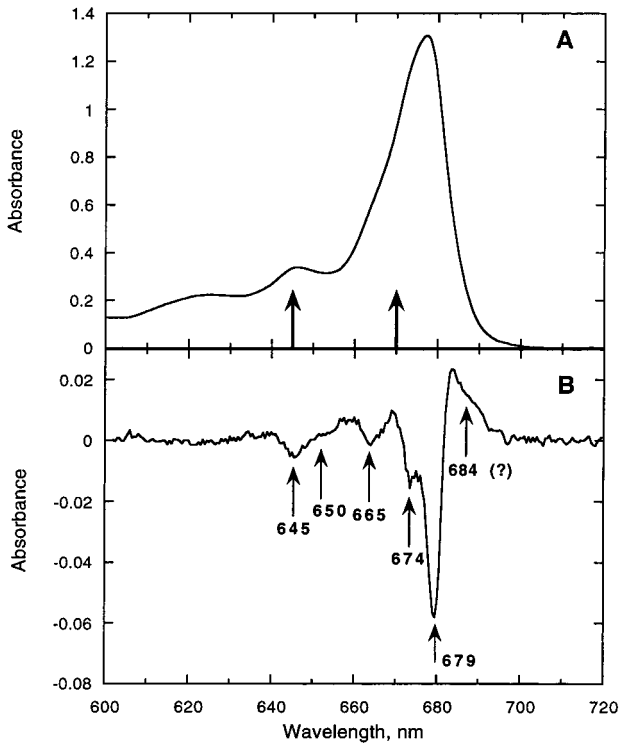

Figure 1. (A) 77 K ground-state absorption spectrum of the Lhca1 subunit of the LHC I peripheral antenna in the region of Q_y absorption bands of Chl *a* and Chl *b*. Arrows indicate excitation wavelengths used in the study. (B) Second derivative of the absorption spectrum. Arrows show spectral forms of Chl *b* (C-645, C-650) and Chl *a* (C-665, C-674, C-679, and C-684).

Results and Discussion

Chl a and Chl b Spectral Forms. The ground-state absorption spectrum of the r-Lhca1 at 77 K is shown in Figure 1. A major absorption band at 679 nm and a smaller peak at 645 nm are due to Q_y transitions of Chl *a* and Chl *b*, respectively. A broad absorption band of composite structure at 620 nm is due to an overlap of vibronic bands and Q_x transition bands of Chl *a* and *b*. In contrast to monomeric pigments in solution, the protein in r-Lhca1 modifies the site energies of Chl *a* and *b* resulting in a series of spectral forms that can be resolved by the second derivative of the absorption spectrum (Figure 1B). Thus, spectral forms C-645 and C-650 are assigned to absorption of Chl *b* molecules, whereas C-665, C-674 and C-679 represent absorption of Chl *a* molecules. Due to the inhomogeneous nature of the absorption spectrum, possible pigment−pigment interactions and the insensitivity of derivative spectroscopy to broad absorption bands, the number of spectral forms does not necessarily correspond to the number of molecules bound to the protein. On the basis of HPLC analysis, the reconstituted Lhca1 subunit used in our experiments binds ∼1.5 Chls *b* and 4.5 Chls *a* per 1 lutein (data not shown).

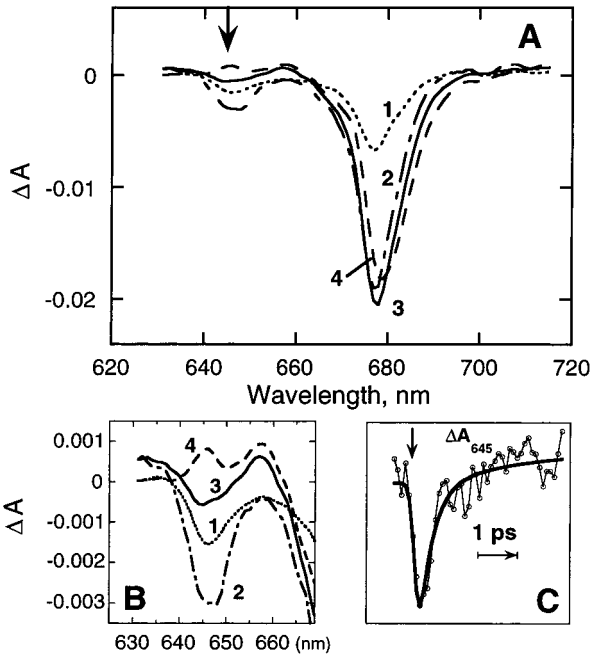

Figure 2. (A) 77 K excitation dynamics in r-Lhca1 monomer induced by excitation of Chl *b* at 645 nm (shown by arrow). Transient absorption spectra in 630−710 nm range (build up of photobleaching) indicate energy transfer from Chl *b* to pools of Chl *a*. (B) Transient absorption changes of the Chl *b* Q_y band scaled up by a factor of 4. Spectra are shown at representative delay times: 0.19 ps (1, dotted line); 0.47 ps (2, dot−dashed line); 1ps (3, solid line); 8.4 ps (4, dashed line). (C) Kinetics of the transient absorption changes at 646 nm. Solid line represents the fit of the kinetics based on the 3-component exponential model (see Figure 3).

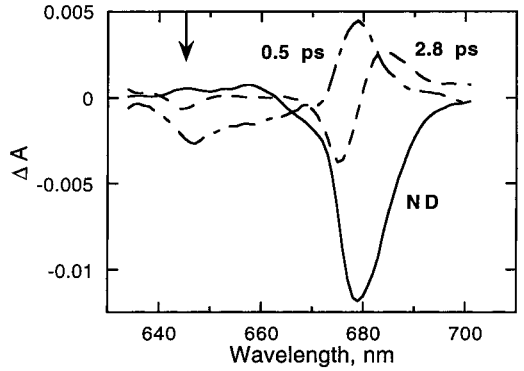

Figure 3. Decay Associated Spectra obtained by global analysis of transient kinetics on the 100 ps time scale with excitation at 645 nm. The resolved kinetic processes are: (1) a 0.5 ps energy transfer from Chl *b*-645 to Chl *a*-679 (DAS shown by dot−dash line); (2) a 2.8 ps process of energy equilibration involving the red most Chl *a*-684 (dashed line); (3) Nondecaying on this time scale process indicating localization of the excitation at 679 nm (solid line).

The 77 K absorption spectrum is similar to the absorption spectrum reported for CP29 (Lhcb4) from PS II[12] reflecting similar Chl *a/b* ratios of these LHCs. However, the Q_y transition band of Chl *b* at 645 nm in r-Lhca1 is 4 nm red shifted relative to the peak of Chl *b* absorption in the CP29, indicating some differences in the environment of Chl *b*.

Excitation Energy Equilibration among Chl b and Chl a Spectral Forms. To detect excitation energy transfer processes between the spectral forms of Chl *a* and Chl *b* we used transient absorption spectroscopy and multicolor excitation of the Chl spectral forms in the 630−680 nm region.

Excitation of Chl *b* Q_y absorption band at 645 nm induces an initial photobleaching at 646−647 nm (Figure 2A and B). The 1−2 nm red shift of the photobleaching peak relative to the excitation wavelength in transient absorption spectra most probably accounts for a contribution of stimulated emission. Spectral evolution of transient absorption bands within 8 ps reveals a complete decay of the ΔA_{646} photobleaching suggesting an energy transfer from Chl *b* molecules to the energy acceptors, which are either pools of Chl *a* or another pool of Chl *b*. The decay of the ΔA_{646} photobleaching is shown in Figure 2C. The excitation dynamics of the Chl *b* absorption band within 8 ps (Figure 2B) might reflect a redistribution of the excitation between C-645 and C-650 pools of Chl *b* (Figure 1). However, a distinct photobleaching around 650 nm in the 8 ps-transient spectrum is overlapped with the excited-state absorption. The details of the excitation dynamics in this region need to be clarified.

In the region of Chl *a* absorption, a prompt photobleaching at 676 nm observed in the 0.19 ps spectrum (Figure 2A) upon excitation at 645 nm most probably indicates direct excitation of Chl *a* via tails of a broad absorption band at 620 nm representing the vibronic bands overlapped with the Q_x transitions of Chl *a* or Chl *b*. A change of $\Delta A_{676}/\Delta A_{646}$ ratio from 4.5 at 0.2 ps to 40 at 1 ps clearly indicates a subpicosecond energy transfer from Chl *b* to Chl *a*. Progressive photobleaching and the 4 nm red shift of the major absorption band in the region of Chl *a* absorption (Figure 2A) illustrate an ongoing process of energy equilibration between pools of Chl *a* (see below).

The spectral evolution of transient ΔA in r-Lhca1 is similar to that reported for CP29 of PSII[12,21] and largely similar to that of r-Lhca4.[16] However, there is a difference in the early transient spectra of r-Lhca4 and r-Lhca1 under excitation of Chl *b*. The $\Delta A_{640}/\Delta A_{679}$ ratio of 0.68 in r-Lhca4 and the $\Delta A_{646}/\Delta A_{679}$ ratio of 0.16 in r-Lhca1 in transient spectra, detected at 470 fs after the excitation, reflect the fact that r-Lhca1 binds fewer Chl *b* molecules per polypeptide. This also may be a result of unresolved ultrafast energy transfer from Chl *b* to Chl *a* in the r-Lhca1.

Global analysis of the transient absorption spectra in Figure 2 resolved at least three kinetic processes in the spectral region of Q_y absorption of Chl *a* and Chl *b* (Figure 3). A 0.5 ps-DAS illustrates the energy transfer process from Chl *b* to Chl *a*. Negative amplitudes of the 0.5 ps-DAS indicate an overlapped decay of excitation in the pools of Chl *b* (C-645 and C-650) as well as pools of Chl *a* at 665 and 670 nm (a broad shoulder in the 660−670 nm region). The process is spectrally unresolved although the pools might be connected either by ultrafast energy transfer or participate in excitonic interactions. Positive amplitude in the 0.5 ps-DAS clearly indicates the absorption wavelength of the energy acceptor, the Chl *a* spectral form at 679 nm (C-679).

The second resolved kinetic process has a lifetime of 2.8 ps (see 2.8 ps-DAS in Figure 3). A decay of the Chl *b* photobleaching around 645 nm shows that some energy equilibration occurs on this time scale within the pools of Chl *b*. The process is not fully resolved because this spectral region is heavily contributed by excited-state absorption of Chl (*a* or *b*) whose rise (positive amplitudes in DAS) spectrally cancels out the excitation decay (negative amplitudes in DAS). The picosecond lifetime of this process suggests the presence of an energy transfer process to a more distant Chl *b* (probably C-650) or Chl *a* molecule absorbing at 684 nm (C-684). The peak of negative ΔA at 676 nm in the 2.8 ps-DAS (Figure 3) indicates that the excited state of Chl *a* in the C-674 pool, which is populated by Chl *b*-Chl *a* subpicosecond energy transfer processes, decays due to an energy transfer process to the Chl

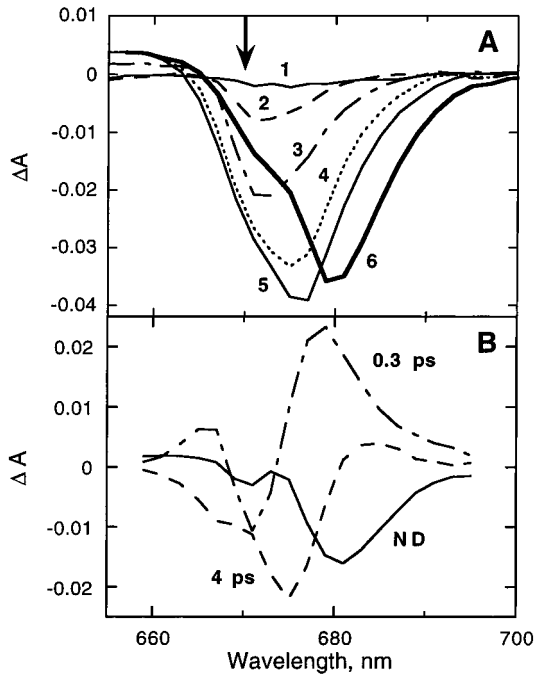

Figure 4. (A) 77 K excitation dynamics in r-Lhca1 monomer induced by excitation of Chl *a* at 670 nm. Transient spectra are shown at representative delay times at 0 ps (1, solid line), 0.19 ps (2, dashed line), 0.38 ps (3, dot−dash line), 0.76 ps (4, dotted line), 1 ps (5, solid line), 3 ps (6, thick solid line). (B) DAS obtained by global analysis of transient kinetics on the 200 ps time scale with excitation at 670 nm. The resolved fast kinetic processes are: (1) a 0.3 ps energy transfer between neighboring Chl *a* molecules (DAS is shown by dot−dash line); (2) a 4 ps equilibration among Chl *a* spectral forms involving Chl *a* at 684 nm (dashed line). This process is similar to 2.8 ps energy transfer in Figure 2. Nondecaying spectrum representing long-lived excited state at low temperature (solid line).

a C-684. This energy transfer process is clearly indicated by the rise of photobleaching at 684 nm. The C-684 Chl *a* spectral form has not been clearly resolved by derivative spectroscopy (indicated by the question mark in Figure 1A).

The nondecaying spectrum in Figure 3 identifies the long-lived excitation state of Chl *a* in the r-Lhca1, which is populated by the 2.8 ps energy transfer process and decays at low temperatures on nanosecond time scale.[16] A peak of the Δ*A* band at 679 nm and a broad shoulder in the 665−670 nm region indicate C-679 and C-665 spectral forms.

To get insight into the energy equilibration processes among Chl *a* spectral forms, we excited the r-Lhca1 at 670 nm with spectrally narrow 200 fs pulses (Figure 4). Spectral evolution of transient absorption within 3 ps clearly indicates ultrafast energy transfer processes within the pools of Chl *a* (Figure 4A). The 0.3 ps-DAS (Figure 4B) reflects the subpicosecond energy transfer processes between neighboring Chl *a* molecules. The inhomogeneously broadened spectral profile (Figure 4A) suggests absorption of several Chl *a* molecules in this spectral region.

The 2.8 ps-DAS in Figure 3 and the 4 ps-DAS in Figure 4B most likely represent similar energy equilibration processes among Chl *a* spectral forms in the r-Lhca1. The 4 ps energy transfer process resolved with excitation of Chl *a* at 670 nm involves the C-665, C-670 and the lower energy shifted spectral form of Chl *a* at 684 nm (C-684) (see 4 ps-DAS in Figure 4B). Energy equilibration processes with similar lifetimes were reported for the CP29.[12] In the Lhca4 subunit, this process involves the longest wavelength absorbing pigments in the LHC I, C-705.[16] The nondecaying spectrum in Figure 4B clearly

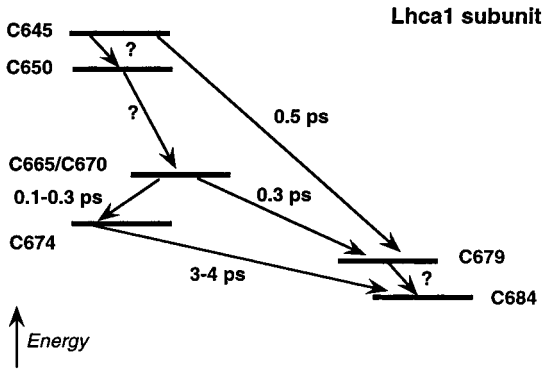

Figure 5. Scheme of energy transfer pathways in the Lhca1 monomer of the LHC I-730 peripheral antenna of Photosystem I. This simplified scheme is based on the assumption that energy transfer flows at 77 K are irreversible and that energy transfer processes between the pools of Chl *b* and Chl *a* represent a sequential kinetic process. C-645 and C-650 are pools of Chl *b*. C-665/C-670, C-674, C-679, and C-684 are pools of Chl *a*. The assignment of spectral forms (particularly that of Chl *b*) is tentative. Question marks indicate unresolved kinetic processes.

indicates that the excitation of the C-670, the C-679, and the C-684 spectral forms stays within the complex for nanoseconds before its decay. However, the 4 nm red shift of the ND spectrum obtained with excitation at 670 nm relative to the ND spectrum obtained with excitation at 645 nm (Figure 3) suggests that the excitation is redistributed toward the localization site at 684 nm, in close agreement with the steady-state fluorescence emission spectrum of the Lhca1 subunit at 77 K.[7]

Scheme of Energy Transfer in the Lhca1 Subunit. The observed processes of energy redistribution among the spectral forms in the absorption spectrum of the Lhca1 subunit suggest a scheme of energy transfer processes based on the global analysis of transient absorption data (Figure 5). The time constant of energy transfer between pools of Chl *b* is unresolved, although there is an indication that part of this process occurs on picosecond time scale (see excitation dynamics in Figure 2). The major pool of Chl *b* (C-645) transfers energy to the major pool of Chl *a* (C-679) within 0.5 ps. The Chl *a* spectral forms C-665/C-670, C-674, and C-679 are also involved in a subpicosecond equilibration. Subpicosecond times of energy transfer between spectral pools of Chl *b* and Chl *a* as well as among the spectral pools of Chl *a* indicate close proximity of some pigments that have spectral contributions into these spectral forms. The 3−4 ps process within Chl *a* spectral pool indicates that the energy equilibration process takes place within pools of C-665/C-670, C-674, and C-684. The excitation localizes on C-679 and C-684 and decays within nanoseconds in the isolated complex.

The sequence of the Lhca1 polypeptide has the closest resemblance with that of the Lhcb4 (CP29) polypeptide.[5,19] For the Lhcb4 polypeptide, a molecular model was constructed based on mutational analysis[10,23] and a structural model of the major LHC II.[6] Both in Lhcb4 and Lhca1, the four central pigment-binding sites located close to the lutein molecules are highly conserved in the protein sequences and most likely bind Chl *a*. In the model of Lhcb4, based on the mutational analysis,[10,21] these sites, termed a1, a2, a4, and a5 according to the labeling by Kühlbrandt et al.,[6] were ascribed to Chl *a* absorbing at 669, 680, 676, and 675 nm, respectively. If one assumes that this structural model is valid for the r-Lhca1, we may ascribe the observed spectral forms C-665, C-670, C-674, and C-679 that are involved in the subpicosecond equilibration, to absorption of four central Chl *a* molecules. It should be noted that the Chl *a* forms C-674, C-679, and C-665 have strong spectral signatures

in both derivative spectra and time-resolved spectra. The attribution of the C-670 to a separate Chl *a* spectral form is more ambiguous because of inhomogeneous broadening of the Chl *a* Q_y transition band. Bassi et al.[10] showed that the pigment-binding sites on the periphery of the Lhcb4 complex might accommodate both Chl *b* and Chl *a* molecules. This might be also the case for the r-Lhca1 because of the uneven number of Chl *a* and *b* molecules ligated to the protein. Observation of two spectral forms of Chl *b*, C-645, and C-650, in steady-state absorption (Figure 1) suggests the presence of at least two Chl *b* molecules in the complex. Presence of the second pool of Chl *b* in the transient absorption spectra is less conclusive (Figure 2B). The excitation dynamics within the Q_y absorption band of Chl *b* does not show clear features of the intraband energy transfer on subpicosecond time scale. This means that the second Chl *b* molecule is located rather far from the first one. Picosecond relaxation of the Q_y band might indicate an energy transfer from Chl *b* C-645 to relatively remote Chl *a* molecules, for example C-684 (see 2.8 ps-DAS in Figure 3). Additional experiments on anisotropy decay are required to address this issue. On the contrary, subpicosecond energy transfer phases that involve the Chl *b* spectral form C-645 and the Chl *a* spectral forms suggest that some of the Chl *a* molecules (most probably C-679 and C-674) are closely located to the Chl *b* molecule. The C-684 Chl *a* spectral form might represent an excitation energy localization site in the r-Lhca1, which is probably located on the periphery of the complex based on the observed picosecond energy transfer times between the C-665/C-670, C-674, C-679, and C-684.

Overall, 2 spectral forms of Chl *b* (C-645 and C-650) and 5 spectral forms of Chl *a* (C-665, C-670, C-674, C-679, and C-684) observed in the derivative spectra (Figure 1) and time-resolved absorption spectra (Figures 2-4) might account for absorption of 2 Chl *b* molecules and 5 Chl *a* molecules, respectively. Four of the Chl *a* molecules possibly occupy the central pigment-binding sites and are in a close contact with two Chl *b* molecules.

The presented assignment of the spectral forms to the pigments in the r-Lhca1 monomer is valid if the pigment−protein interactions dominate the spectral heterogeneity. This situation was suggested for the CP29 that binds 6 Chl *a*, two Chl *b* and two central xanthophyll molecules.[10] In this case, the observed absorption spectral forms can be largely explained by pigment−protein interactions. In contrast, the Lhca4 complex has clear evidence for strong pigment−pigment interactions in the long-wavelength absorbing chlorophylls.[18]

The following arguments could complicate the direct assignment presented above. The observed subpicosecond and picosecond phases of the excitation relaxation within the inhomogeneously broadened Chl *a* and *b* Q_y transition bands may represent some kinetic heterogeneity between different complexes in the sample. Additionally, the possibility of stronger pigment−pigment interactions in the Lhca1 subunit associated with the appearance of new absorption bands cannot be ruled out. A detailed mutational analysis of the pigment-binding sites in the Lhca1, an analysis of absorption changes due to the mutations and a kinetic modeling are needed for the decisive assignment of the observed spectral forms to specific pigments.

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