Carotenoids in Energy Transfer and Quenching Processes in Pcb and Pcb−PS I Complexes from *Prochlorothrix hollandica*

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Chlorophyll (Chl) *a*/*b*-binding proteins from *Prochlorothrix hollandica* known as Pcb antennae were studied by femtosecond transient absorption technique to identify energy transfer rates and pathways in Pcb and Pcb−PS I complexes. Carotenoids transfer energy to Chl with low efficiency of ∼25% in Pcb complexes. Interestingly, analysis of transient absorption spectra identified a pathway from the hot S_1 state of zeaxanthin and/or β-carotene as the major energy transfer channel between carotenoids and chlorophylls in Pcb whereas the S_2 state contributes only marginally to energy transfer. Due to energetic reasons, no energy transfer is possible via the relaxed S_1 state of carotenoids. The low overall energy transfer efficiency of carotenoids recognizes chlorophylls as the main light-harvesting pigments. Besides Chl *a*, presence of Chl *b*, which transfers energy to Chl *a* with nearly 100% efficiency, significantly broadens the spectral range accessible for light-harvesting and improves cross section of Pcb complexes. The major role of carotenoids in Pcb is photoprotection.

Introduction

Green oxyphotobacteria, including genus *Prochloron*, *Prochlorococcus*, and *Prochlorothrix*, are photosynthetic prokaryotes that are classified as cyanobacteria. Contrary to traditional cyanobacteria that utilize phycobilisomes to collect light, the green oxyphotobacteria have developed a light-harvesting strategy that relies on three types of specific membrane-bound Chl *a*/*b*-binding proteins, the Pcb (prochlorophyte Chl *a*/*b*-binding) proteins (PcbA, PcbB, and PcbC).[1−4] These antenna proteins belong to a phylogenetic group containing the iron-stress-induced *isiA* gene product of cyanobacteria,[5] and CP43, photosystem (PS) II core antenna protein.[6] However, contrary to the CP43 protein, the IsiA and Pcb proteins were shown to exist in multiple copies that usually form an antenna ring around the trimeric PS I,[7,8] or a layer attached to the PS II dimer.[9] While the IsiA protein binds only Chl *a*, the Pcb antennae isolated from green oxyphotobacteria contain both Chl *a* and Chl *b*. The 18-mer of Pcb proteins forming a ring around PS I trimer was found in *Prochlorococcus marinus*[8] and *Prochlorothrix hollandica*.[10]

Although multiple copies of IsiA and Pcb proteins greatly enhance the light-harvesting capacity,[11,12] details of energy-transfer processes have been studied only for the IsiA proteins. In a femtosecond transient absorption experiment, Melkozernov et al.[13] showed that energy transfer from IsiA proteins to PS I core in *Synechocystis* takes place on a time scale of a few picoseconds. A time constant of 2 ps was found for the same process in *Synechococcus*,[14] but application of global analysis and kinetic modeling of transient absorption data have allowed to disclose a complicated network of processes. It was shown that equilibration between the IsiA proteins, energy transfer from IsiA to the PS I core, and back transfer from the PS I core to the IsiA proteins occur at comparable time scales.[14] Importantly, both studies showed that the overall trapping time in the IsiA−PS I supercomplex is about twice longer than that in the PS I core (∼40 and ∼20 ps, respectively), reflecting doubling of antenna size in the IsiA−PS I supercomplex.[13,14]

So far, no time-resolved studies have been carried out on Pcb proteins, in which the light-harvesting capacity is also enhanced as in the IsiA antenna ring, but the presence of Chl *b* in Pcb antenna further complicates the network of energy-transfer pathways. Similarly, the role of carotenoids in energy transfer remains unresolved in Pcb proteins. In various light-harvesting systems, carotenoids transfer energy to chlorophylls from their two lowest excited states, the strongly allowed S_2 state and the lowest forbidden S_1 state.[15] In the past few years, additional energy-transfer channels were found[16] occurring either via hot S_1 state or the so-called S* state, whose origin still remains to be determined.[17] Very recently, energy transfer between carotenoids and Chl *a* was identified in IsiA complexes.[18] Both Pcb and IsiA proteins contain four carotenoids. The IsiA protein binds two molecules of β-carotene, one zeaxanthin and one echinenone.[19] Two β-carotenes and one zeaxanthin were found also in the Pcb protein, but echinenone is replaced by α-carotene in Pcb.[20] In IsiA, carotenoids transfer energy exclusively from the S_2 state, but with low efficiency of 22% (β-carotene) and 37% (echinenone). Comparable results were obtained for the closest relative of the IsiA and Pcb proteins, the CP43 protein containing four β-carotenes[21,22] that transfer energy to Chl *a* from the S_2 state with an efficiency of

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~30%.[23] In addition, a minor channel via hot S_1 state was proposed by de Weerd,[24] but no energy transfer via the relaxed S_1 state was identified so far.

Besides the light-harvesting function, carotenoids in photosynthetic antenna also play the role of protective agents by quenching Chl excited states upon excess light conditions. The exact mechanism of the quenching is still a matter of considerable debate. Two basic mechanisms involving direct interaction between carotenoids and chlorophylls were proposed: electron-transfer quenching[25] and energy-transfer quenching.[26,27] While no study of Pcb-containing systems was carried out so far, it is known that quenching certainly exists in isolated IsiA proteins, because Chl fluorescence lifetimes in IsiA are significantly shortened as compared with Chl in solution.[19] Recent studies of the quenching mechanism in the IsiA proteins of cyanobacteria have suggested that the carotenoid echinenone directly quenches the excited states of Chl *a* via energy transfer from the Q_y state of Chl *a* to the S_1 state of echinenone.[28]

Three different types of Pcb proteins were found in *Prochlorothrix hollandica*: PcbA, PcbB, and PcbC,[29] but the ring around the PS I trimers is formed exclusively by the PcbC proteins. Recently, PcbC proteins from *P. hollandica* were isolated and characterized,[20] offering a possibility to study excited-state processes in these proteins and compare them with the complete antenna system of *P. hollandica*, the Pcb−PS I supercomplex consisting of PS I trimer surrounded and 18 PcbC proteins that form a ring around the PS I trimer.[10] The data presented here are restricted to PcbC protein that will be hereafter denoted as Pcb.

Here we show the results of femtosecond transient absorption spectroscopy that aim to disclose the role of carotenoids and chlorophylls in energy transfer in isolated Pcb proteins and Pcb−PS I supercomplexes. The data clearly demonstrate that the light-harvesting process in these complexes is primarily provided by Chl *a* and Chl *b* molecules, whereas the carotenoids play rather a minor role in the antenna function. On the other hand, carotenoids are the key photoprotective pigments regulating the energy flow within the Pcb−PS I supercomplex.

Materials and Methods

Pcb proteins and Pcb-PS I supercomplexes were prepared from *P. hollandica* as described previously.[20] The fractions obtained from sucrose gradient were concentrated to yield optical density ~0.2 at 495 nm in a 1 mm cuvette. Absorption spectra were measured on UV-300 (Spectronic Unicam, Cambridge, UK) spectrophotometer; fluorescence spectra were recorded on Fluorolog 2 (Spex, USA) spectrofluorometer. A 1 mm path length quartz cuvette was used for steady-state absorption; low-temperature emission spectra were measured in a homemade 1 mm cuvette designed for measurements at 77 K.[30] By varying the sample concentration, we have tested that the concentration of samples for fluorescence spectroscopy was always low enough to prevent reabsorption.

Transient absorption spectra were measured at room temperature using a femtosecond spectrometer employing Ti:sapphire amplifier (Intregra-i, Quantronix) as a primary source of femtosecond pulses. Excitation pulses were generated in an optical parametric amplifier (TOPAS, Light Conversion), while a white-light single filament continuum generated in a 2 mm sapphire plate was used as a probe. The mutual orientation of the excitation and probe beams polarization was set to the magic angle (54.7°). A 1 mm path length rotating quartz cuvette spinning at a rate to ensure that each excitation pulse hit a fresh sample was used for transient absorption measurements. Time-resolved absorption changes were measured in a broad spectral range from 474 to 716 nm by detecting the dispersed white light by double-diode array after excitation with ~130 fs laser pulses centered at either 495 nm (carotenoid excitation) or 643 nm (Chl *b* excitation). By use neutral-density filters, intensity of excitation was kept at ~6.0 (λ = 495 nm) or 8 (λ = 643 nm) × 10^{13} photons $pulse^{-1}$ cm^{-2}.

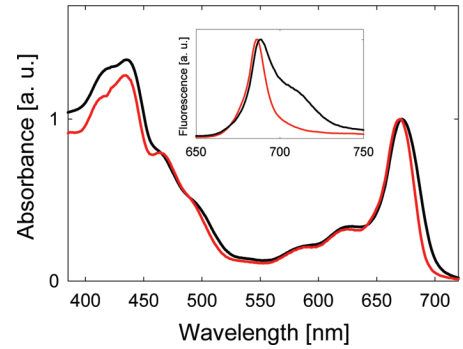

Figure 1. Absorption spectra of Pcb complex (red) and Pcb−PS I complex (black). Inset: Low-temperature fluorescence spectra of the complexes. Both absorption and fluorescence spectra are normalized at the Q_y band of Chl *a*.

The absorption kinetics collected by diode-array detectors were fitted globally. To visualize the excited-state dynamics, we assume that the excited Pcb complexes evolve according to a sequential, irreversible scheme A → B, B → C, C → D, ... Time constants of these processes correspond to lifetimes of the species A, B, C, D, ... The spectral profile of the species is called evolution-associated difference spectrum (EADS). It must be noted that EADS extracted from the sequential model do not correspond to individual excited-state species in a complex system such as the Pcb−PS I supercomplex studied here. Instead, they will inevitably contain a mixture of excited-state species. Yet, EADS help to visualize excited-state processes and carry important information about excited-state dynamics.[31]

Results

Room temperature absorption spectra of Pcb proteins and the Pcb−PS I supercomplex are shown in Figure 1. They are dominated by a characteristic structure of Chl *a* absorption consisting of the Soret band at 437 nm, and the Q_y band at 671 nm. For the Pcb−PS I complex, the Q_y absorption is broadened toward lower energies, extending beyond 700 nm, reflecting the absorption of the PS I core. The presence of Chl *b* in Pcb antenna is demonstrated by its characteristic Soret band peaking at 465 nm, and weak Q_y absorption shoulder around 645 nm. The spectral bands between 560 and 640 nm are due to higher vibrational Q_y and/or Q_x bands of Chl *a*. The spectral band centered around 495 nm in all complexes is due to the lowest, 0−0 vibrational band of the S_0−S_2 transition of carotenoids while higher vibrational bands of carotenoids overlap with the Soret band of Chl *b*.[20] Consequently, the 495 nm absorption band could be used for selective excitation of carotenoids in Pcb and Pcb−PS I complexes. For better characterization of the complexes, we have measured fluorescence spectra at 77 K (Figure 1, inset). Isolated Pcb antenna can be clearly identified by narrow emission band at 685 nm. For the Pcb−PS I supercomplex, the emission maximum is at 689 nm, and additional band centered around 710 nm is due to PS I emission.[10,20]

Transient absorption spectra of both samples recorded at 1 ps after excitation of the carotenoid S_2 state at 495 nm are shown

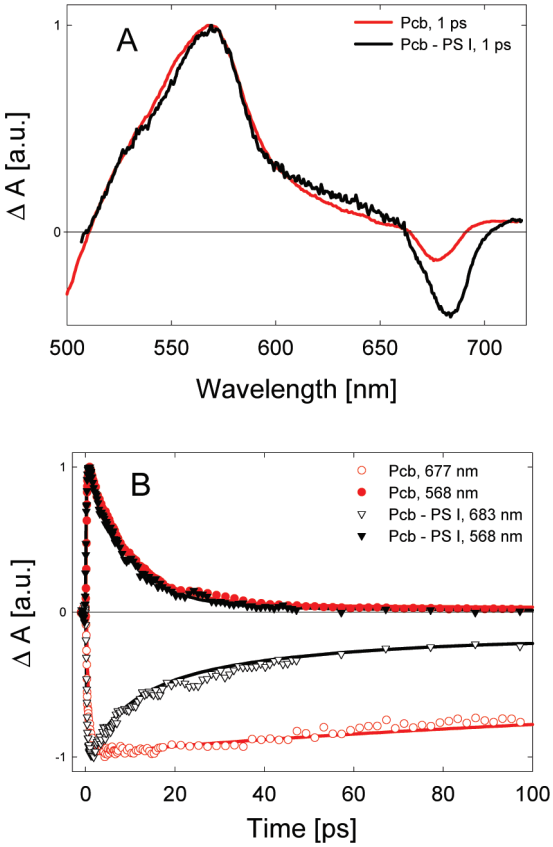

Figure 2. (A) Transient absorption spectra of Pcb (red) and Pcb−PS I (black) complexes recorded at 1 ps after excitation at 495 nm. (B) Kinetics measured at 568 nm (full symbols), and at maxima of the Chl *a* bleaching (open symbols). Solid lines are fits. Transient absorption spectra and kinetics are normalized to maximum.

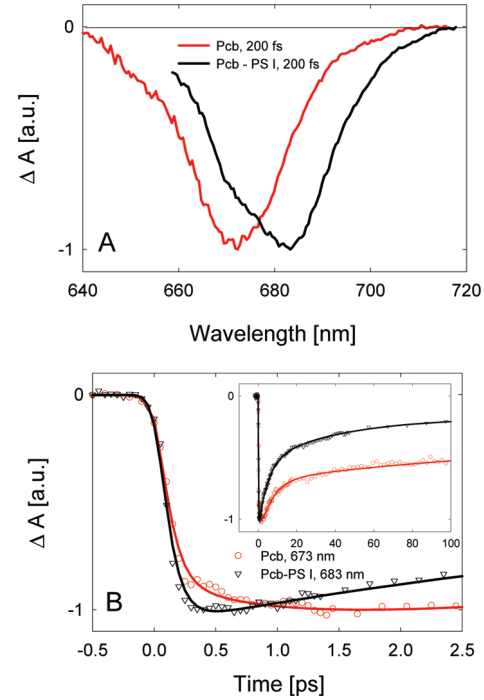

Figure 3. (A) Transient absorption spectra of Pcb (red) and Pcb−PS I (black) complexes recorded at 200 fs after excitation of Chl *b* at 643 nm. (B) Kinetics of Chl *a* rise measured at the maxima of Chl *a* bleaching at 673 nm (Pcb) and 683 nm (Pcb−PS I). Inset shows kinetics at longer time scale. Solid lines are fits.

in Figure 2A. At this time delay, the S_2-S_1 internal conversion is finished and the excited-state absorption band, peaking at 568 nm, is due to the S_1-S_n transition of carotenoid. The negative signal below 510 nm is due to the ground-state bleaching of carotenoids, while the negative band at 677 nm reflects the ground-state bleaching of Chl *a* in Pcb antenna, indicating that energy transfer occurs between carotenoid and Chl *a*. Transient absorption spectra of Pcb and Pcb−PS I complexes are essentially identical in the carotenoid spectral region, reflecting the spectroscopic identity of β-carotene (occurring in both Pcb and PS I) and zeaxanthin (occurring exclusively in Pcb). The identity of the transient absorption spectra also confirms that, if a fraction of α-carotene is present in the Pcb antenna,[20] these molecules are not excited at 495 nm. The major difference between the two samples is the position and magnitude of the Chl *a* bleaching signal. The Chl *a* bleaching peaks at 677 nm in Pcb and at 683 nm in the Pcb−PS I supercomplex, mirroring the shift of the Q_y band in the absorption spectra (Figure 1). The difference in magnitude is related to less efficient energy transfer from carotenoids to Chl's in isolated Pcb complexes (see Discussion section).

Kinetics recorded at the maxima of most prominent transient absorption bands are shown in Figure 2B. The decay probed at 568 nm, corresponding to the peak of the S_1-S_n band and monitoring the S_1 lifetime of carotenoids, is identical in both samples. Global fitting (see below) reveals the S_1 lifetime of ~9 ps, which is close to the known S_1 lifetimes of zeaxanthin and β-carotene in solution.[15,32,33] In the Chl *a* bleaching region, excited Chl *a* in Pcb monitored at 677 nm does not decay substantially within the 100 ps window. A more complex dynamics is, however, detected in the Pcb−PS I supercomplex. Chl *a* decay at 683 nm is multiexponential, featuring time components on a time scale of picoseconds. These additional components characterize processes associated with energy transfer from Pcb to the PS I core and equilibration and trapping within the PS I core.[13,14]

To test the role of Chl *b* in energy transfer, we have excited both samples at 643 nm, a wavelength that corresponds to a weak shoulder attributed to Chl *b* (Figure 1). In order to estimate how much of excitation light is absorbed by Chl *b* at 643 nm, we have used comparison of absorption spectra of Pcb, which has Chl *b*:Chl *a* ratio of 1:4,[20] and IsiA, which does not contain Chl *b*. Then, assuming that wavelength of the Chl *b* Q_y maximum, and Chl *a* and Chl *b* extinction coefficients are in Pcb the same as in LHCII complex, we have estimated that approximately 45% of photons at 643 nm is absorbed by Chl *b*. Thus, even at 643 nm we still predominantly excite Chl *a*, but it is the wavelength where the fraction of excited Chl *b* is the largest. In the Pcb−PS I complex, most of the 643 nm photons are captured by Chl *a*, because the Chl *b*:Chl *a* ratio is 1:7 in this complex.[20] Already at 200 fs (Figure 3A), transient absorption spectra of both samples exhibit feature typical of Chl *a* bleaching, which is mainly the result of direct excitation of Chl *a*. However, closer inspection of kinetics recorded at the respective maxima of the Chl *a* bleaching bands (Figure 3B) reveals a rise component in Pcb complex that is identified as due to Chl *b* to Chl *a* energy transfer.

In order to extract further details about excited-state and energy-transfer processes, a global fitting analysis has been applied to the transient absorption data. Fitting results are visualized as evolution-associated difference spectra (EADS). A minimum of four time components was needed to fit excitation dynamics of the Pcb complex after carotenoid excitation (Figure 4A). The first EADS represents a spectrum of the initially excited species and has a typical shape of the S_2

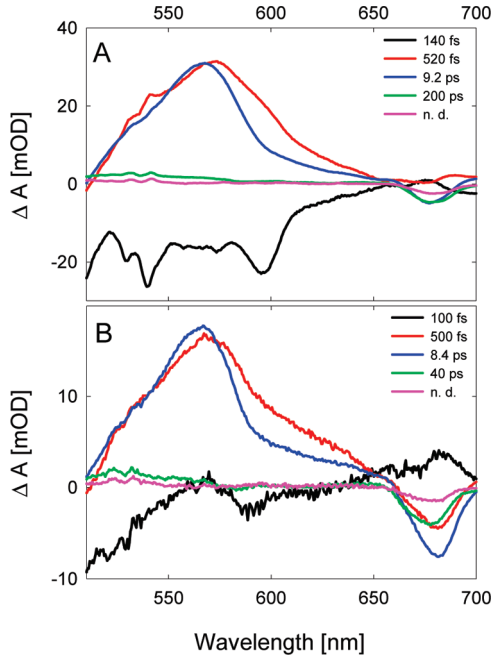

Figure 4. EADS extracted from global fitting of data obtained after 495 nm excitation of Pcb (A) and Pcb−PS I (B) complexes. n.d.: nondecaying component that does not decay within the time window of our experiment.

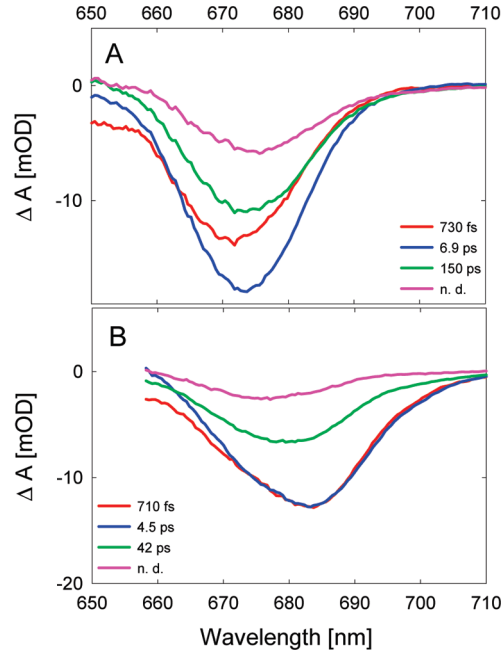

Figure 5. EADS obtained from global fitting of data recorded after excitation of Pcb (A) and Pcb−PS I (B) complexes at 643 nm. n.d.: nondecaying component that does not decay within the time window of our experiment.

state spectrum of a carotenoid. This EADS decays within 140 fs to form the second EADS that is dominated by a broad positive signal characteristic of a carotenoid hot S_1 state.[34,35] A minor dip around 675 nm indicates presence of the Chl *a* bleaching in the second EADS, suggesting that part of the energy transfer proceeds via the S_2 pathway. The EADS attributed to the hot S_1 state further decays in 520 fs to form EADS typical of the relaxed S_1 state. Thus, the 520 fs component is attributed to vibrational relaxation in the S_1 state. It must be noted that during the transformation from the second to third EADS there is also a significant gain of Chl *a* bleaching. Further evolution occurs with a 9.2 ps component that characterizes the S_1 lifetime. Since this lifetime matches that of zeaxanthin and β-carotene in solution,[32,33] and since there is no further increase of Chl *a* bleaching signal, it is clear that no energy transfer occurs from the S_1 state of carotenoids in the isolated Pcb antenna. The slow Chl *a* bleaching recovery is characterized by a 200 ps component, but it must be noted that time window used in our experiment precludes precise determination of this time component. When Chl *a* bleaching region is fitted separately (Figure S2, Supporting Information), the best fit is obtained with the slow decay having a time constant of 120 ps. Even with this error margin, the Chl *a* bleaching recovery in Pcb is slower than ∼70 ps observed for corresponding decay in IsiA.[18]

Very similar time constants were extracted from the global fitting of the data recorded for the Pcb-PS I supercomplex (Figure 4B), except the slowest component have a lifetime of 40 ps. The first EADS decays in 100 fs to form the spectrum of the hot S_1 state, but, contrary to the Pcb complex, the second EADS contains substantial Chl *a* bleaching signal that is indicative of more efficient S_2-mediated energy transfer in the Pcb-PS I supercomplex. The hot S_1 state decays within 500 fs, and the formation of the third EADS characterizing the relaxed S_1 state is again accompanied by an increase of the Chl *a* bleaching at 681 nm. The lifetime of the third EADS (the S_1 lifetime) is 8.4 ps. The next EADS, decaying with a 40 ps time constant has no contribution from carotenoids and is therefore due solely to equilibration and trapping processes among Chl *a* molecules within the PS I core.[13,14] Since the 8.4 ps component, assigned to the carotenoid S_1 lifetime, also significantly contributes to Chl *a* bleaching recovery, we have applied global fitting to carotenoid spectral region (500−650 nm) and Chl *a* bleaching region (650−720 nm) separately. Results, shown in Figure S2 (Supporting Information), confirms the S_1 lifetime of carotenoids, but if the Chl *a* bleaching region is fitted separately, the third EADS has a lifetime of 5.7 ps, indicating that this component is not related to carotenoid dynamics, but it is due solely to Chl *a* equilibration and/or annihilation as described in Discussion section.

Global fitting the data measured after excitation of Chl *b* at 643 nm (Figure 5) provides initial EADS that contains significant amount of Chl *a* bleaching, indicating direct excitation of Chl *a* at 643 nm. In Pcb, however, the first EADS contains a distinct bleaching below 660 nm attributable to Chl *b*. Further evolution is accompanied by disappearance of the Chl *b* bleaching and concomitant increase of Chl a signal 673 nm. This process occurs with a time constant of 730 fs. In Pcb−PS I complex the initial EADS suffers from scattering below 660 nm, but a hint of Chl *b* bleaching, which again disappears with ∼700 fs time constant, is observed even for this complex. Contrary to Pcb, however, the transition between the first and second EADS is not accompanied by increase of the Chl *a* signal. Thus, while in Pcb the 730 fs time component can be attributed to Chl *b* to Chl *a* energy transfer, in Pcb−PS I this process is most likely masked by due to significantly larger amount of Chl *a* excited at 643 nm in Pcb−PS I. This is in agreement with the kinetics shown in Figure 3B; while the kinetics monitoring the Chl *a* rise in Pcb contains a distinct slow component, no such component is observed for Pcb−PSI.

Further evolution of the excited-state dynamics reflects equilibration processes among the Chl *a* molecules. In the isolated Pcb antenna, the second EADS generated via energy transfer from Chl *b* decays with a time constant of 6.9 ps. This time component is missing in data obtained after excitation of

carotenoid. To determine the origin of this component, we have measured kinetics with different excitation intensities (Figure S3, Supporting Information). While the kinetics measured after 490 nm excitation do not show any dependence on excitation intensity, kinetics obtained with excitation at 643 nm exhibit significant differences. Amplitude of the 6.9 ps component decreases with decreasing excitation intensity, suggesting this decay component is due to annihilation. The fact that annihilation is not present after 490 nm is likely due to low-efficiency energy transfer that prevents existence of more than one excited Chl *a* molecule within one Pcb complex, and thus annihilation cannot occur after carotenoid excitation at excitation intensities used here. EADS corresponding to the 6.9 ps component decays within 150 ps to form the final EADS that does not decay within the time frame of our experiments. The final, nondecaying EADS has the most red-shifted Chl *a* bleaching, suggesting that excitation energy resides at the lowest energy Chl *a* that does not transfer energy any further.

Similar pattern is obtained for the Pcb−PS I complex, except the second and third EADS have lifetimes of 4.5 and 42 ps, respectively. Nondecaying EADS in Pcb−PS I is not only much weaker than that for Pcb, but it is also significantly blue-shifted (peaking at 676 nm) as compared with the Chl *a* bleach in the second and third EADS in Figure 5B. Because the bleaching maximum of the final EADS is close to that observed in isolated Pcb, the final EADS in the Pcb−PS I complex most likely corresponds to a fraction of free Pcb proteins.

Discussion

The purity and composition of the samples used in this study was discussed previously.[20] Thus, we will only briefly mention details important for interpretation of our spectroscopic data. Both absorption and emission spectra demonstrate that Pcb does not contain any residual PS I or free pigments that could hamper the transient absorption data. Similarity of transient absorption spectra measured for Pcb and Pcb−PS I complexes shown in Figure 2 demonstrates that the carotenoids contributing to the transient signal in Pcb must have similar spectroscopic properties as β-carotene which is the only carotenoid in PS I.[36,37] Therefore, we conclude that β-carotene and zeaxanthin, both present in Pcb complexes,[20] are the only carotenoids excited at 495 nm. α-Carotene, which lacks one conjugated double bond and is thus expected to have blue-shifted absorption spectrum, does not contribute to the transient absorption signals and can be therefore ignored in our analysis.

The residual signal obtained after excitation of the Pcb−PS I complex indicates that the sample contains some fraction of free Pcb complexes that cannot transfer energy to the PS I core. The magnitude of the residual bleaching suggests that the fraction of the free Pcb complexes does not exceed 20%. A more complicated task is to quantify the fraction of free PS I complexes. To estimate if the sample can contain a significant fraction of free PS I complexes, we have compared absorption, emission, and transient absorption data of Pcb−PS I complexes with those recorded for PS I-only sample (Supporting Information, Figure S1). Both absorption and emission spectra of PS I-only samples are red-shifted from the respective maxima in Pcb−PS I complexes, which is the primary indicator of intactness of the Pcb−PS I[10,20] or IsiA−PS I[14] complexes. Another marker is the presence of the 40 ps component in the Pcb−PS I complexes. This component has been observed earlier in the IsiA−PS I complexes and was assigned to the trapping time that is twice longer than in PS I-only samples. Since the same situation occurs here when comparing Pcb−PS I and PS I-only complexes (Supporting Information, Figure S1), we can safely conclude that dominant fraction of our sample indeed consists of full Pcb−PS I complexes.

Energy Transfer in Pcb Complexes. Evidence for energy transfer between carotenoids and Chl is obtained from the transient absorption experiment. Excitation at 495 nm excites almost exclusively the lowest absorption band of carotenoids, and appearance of the Chl *a* bleaching within the first few hundred femtoseconds is a clear evidence of carotenoid−Chl energy transfer. Yet, the magnitude of the Chl *a* bleaching in the transient absorption spectra indicates that efficiency of energy transfer is low. Providing that extinction coefficient of S_1-S_n transition is comparable to that of S_0-S_2 transition, which is a reasonable approximation for β-carotene and zeaxanthin,[33] comparison of magnitudes of S_1-S_n transition and Chl *a* bleaching with corresponding ground-state absorption bands suggests that overall energy transfer efficiency does not exceed 25%.

The energy-transfer efficiency around 25% classifies the Pcb protein among the antenna systems with the least efficient carotenoid energy donors. It is comparable with overall carotenoid−Chl energy transfer efficiency in two close relatives of Pcb, IsiA and CP43, in which carotenoids transfer energy to Chl *a* with ∼30% efficiency,[18,24] indicating that the low carotenoid−Chl efficiency is likely a common feature of this family of antenna proteins. Interestingly, however, contrary to CP43 or IsiA that utilize almost exclusively the S_2 pathway,[18,23,24] global fitting analysis of the transient absorption data recorded here suggests different energy transfer pathways in the isolated Pcb proteins.

Although the pigment composition of Pcb is more complex than that of CP43, the lifetimes of the first three EADS of 140 fs, 0.52 ps, and 9.2 ps extracted from global fitting analysis of the isolated Pcb proteins (Figure 4A) are comparable to those obtained for CP43 (70 fs, 0.4 ps, and 9 ps)[24] or IsiA (66 fs, 0.6 ps, and 7.6 ps),[18] suggesting similar excited-state dynamics in both complexes. Yet, a closer inspection of the EADS reveals significant difference. In CP43 and IsiA, the second EADS contains a fully developed Chl *a* bleaching that does not grow any further.[18,24] This is compelling evidence that the energy transfer in CP43 and IsiA occurs predominantly from the S_2 state of carotenoid, as also evidenced by the shorter lifetime of the first EADS in CP43 and IsiA. In Pcb, however, the second EADS contains only weak dip at 675 nm, while the fully developed Chl *a* bleaching occurs only in the third EADS that is formed with 520 fs time constant (Figure 4a). This would imply that the S_2 pathway accounts only marginally to the overall energy transfer. Instead, the major part of energy transfer occurs during the process characterized by the time constant of 520 fs that is usually associated with relaxation of the hot S_1 state of carotenoid.[34,35] The relaxed S_1 state does not serve as energy donor, because the EADS has a lifetime of 9.2 ps, matching the S_1 lifetime of β-carotene and zeaxanthin in solution.[33]

Analysis of EADS thus points to the hot S_1 state as the major energy donor in Pcb complex. Yet, other possible explanations should be discussed. First, the observed results could be explained by energy transfer from the carotenoid S_2 state that is relayed to Chl *a* via Chl *b*. In such case, the formation of the Chl *a* bleaching would be associated with energy transfer from Chl *b* to Chl *a*. The time constant of Chl *b* to Chl *a* energy transfer is 730 fs (Figure 5A), thus longer than the 520 fs component characterizing the Chl *a* rise after 495 nm excitation. In order to test reliability of the time components obtained from

global fitting the whole data set, we have fitted the spectral regions of carotenoid S_1-S_n transition and Chl *a* bleaching separately. If the rise of Chl *a* bleaching were due to relaying energy via Chl *b*, the time constant obtained from separate fitting of the Chl *a* region should be due to Chl *b* to Chl *a* energy transfer, while in the carotenoid S_1-S_n region it would reflect the relaxation of the hot S_1 state. Results of the separate global fitting are shown in Figure S2 (Supporting Information). The lifetime of the second EADS in the carotenoid region is almost the same (580 fs vs 520 fs obtained from fitting the whole data set), but the major component of the Chl *a* rise is slightly shortened to 410 fs. Thus, although the hot S_1 decay and Chl *a* rise are somehow different when global fitting is applied separately to carotenoid and Chl *a* regions, it is clear that the appearance of Chl *a* is not due to energy transfer from Chl *b*. If this were the case, we would expect the corresponding time constant to be prolonged to match the 730 fs time constant of Chl *b* to Chl *a* transfer. The same situation occurs for Pcb−PS I complex (Figure S2, Supporting Information). Consequently, relaying energy transfer via Chl *b* is unlikely.

The second possibility is that the red part of the S_1-S_n transition pronounced in the second EADS is not due to a hot S_1 state, but rather due to a relaxed S_1 state of a specific carotenoid whose binding site shifts the S_1-S_n transition to lower energies. In that case, the 520 fs component would correspond to the lifetime of the relaxed S_1 state shortened by energy transfer from this specific carotenoid. However, this presumed red-shift would lower the S_1 energy, making the efficient energy transfer from the relaxed S_1 state very unlikely. Thus, we conclude that most of the energy absorbed by carotenoids, β-carotene and zeaxanthin, in the isolated Pcb is likely transferred to Chl *a* via hot S_1 state. Although utilization of the hot S_1 pathway was identified in other light-harvesting complexes (LH2,[38,39] LHCII[40]) including CP43,[24] it has never accounted for more than 10% of the total carotenoid-mediated energy transfer, and the dominant pathway proceeded usually via the S_2 state.[23] In Pcb, however, the situation is reversed. The S_2 channel is only marginal, while the energy transfer from the hot S_1 state becomes dominant.

Excitation of the isolated Pcb at 643 nm provides evidence for very efficient energy transfer from Chl *b* to Chl *a*. The Chl *b* transfers energy to Chl a with 730 fs time constant, implying that Chl *b* transfers energy to Chl *a* essentially with 100% efficiency.

Energy Transfer in Pcb−PS I Complexes. Excitation of the whole Pcb−PS I complex consisting of the PS I core trimer surrounded by multiple copies of Pcb proteins adds to the complexity of energy-transfer pathways. Although the first three EADS have essentially the same lifetimes as for the isolated Pcb, yielding 100 fs, 0.5 ps, and 8.4 ps, their spectral shapes differ. First, it is obvious that substantial amount of energy is transferred directly from the S_2 state, as documented by the significant increase of the Chl *a* bleaching during the first, 100 fs, step. This additional S_2 pathway is associated with β-carotene molecules located in the PS I core that are known to efficiently utilize the S_2 pathway.[41] During the 0.5 ps step, further increase of the Chl *a* bleaching suggests another energy-transfer channel that is identified as the hot S_1 channel occurring in the Pcb proteins. The 8.4 ps time constant associated with the relaxed S_1 state again points to no energy transfer via the relaxed S_1 state of carotenoids.[14] It should be noted that application of global fitting analysis separately to the Chl *a* bleaching region (Figure S2, Supporting Information) resulted in time constant of 5.7 ps instead of 8.4 ps associated with the third EADS, setting an error range of this component. Thus, within 1 ps after excitation of carotenoids in the Pcb−PS I complex, energy is transferred to chlorophylls. The subsequent dynamics is therefore related to energy transfer and equilibration among the chlorophyll molecules.

The time constants related to Chl *a* dynamics obtained here agree with those reported for the IsiA−PS I complexes.[13] The 5.7−8.4 and 40 ps time constants correspond well to the 6.6 and 38 ps components in the IsiA−PS I complex of *Synechococcus*,[14] or to 10 and 44 ps obtained for the same complex from *Synechocystis*.[13] It is also important to note that these two components are independent of excitation intensity (Figure S3, Supporting Information) and thus they are due to intrinsic dynamics which is not affected by annihilation. In line with the conclusions about the IsiA−PS I complexes, the 5.7−8.4 ps component most likely encompasses multiple processes: relaxation within the chlorophyll pool in the PS I core, and energy transfer from the Pcb ring to the PS I core.[13,14] Thus, although we could not resolve the intrinsic rate constant for energy transfer between Pcb and PS I core, our results provide evidence that energy captured by the Pcb ring reaches the PS I core on a time scale of a few picoseconds. As for the IsiA−PS I complex,[13,14] we assign the 40 ps component to trapping in the reaction center. Comparison of Pcb−PS I kinetics with those recorded for the PS I complexes isolated from *P. hollandica* (Supporting Information, Figure S1) suggests that trapping time is about twice faster in the isolated PS I complexes, indicating that antenna size of the Pcb−PS I complex is doubled as compared with the PS I-only system. The same trend was reported also for the IsiA−PS I complexes of cyanobacteria,[13,14] demonstrating that the Pcb antenna ring of *P. hollandica* functions in very similar way as the IsiA ring of cyanobacteria.

Quenching Processes in Pcb Complexes. Besides the complicated network of energy transfer, the ~200 ps decay component associated with lifetime of the final acceptor in Pcb complexes indicates that quenching of Chl *a* excited states must also take place in Pcb. This observation is in good agreement with time-resolved fluorescence data on IsiA complexes, which exhibited a comparable fluorescence lifetime.[19] Somewhat shorter (<100 ps) Chl *a* lifetime was found in IsiA in transient absorption experiments.[18,28] While this component is observed regardless of the excitation wavelength (fitting gives 160 ps after 643 nm excitation), excitation at 643 nm opens an additional Chl *a* decay channel characterized by a time constant of ~7 ps (Figure 5A). Since amplitude of this decay component depends on excitation intensity (Supporting Information, Figure S3), we attribute this component to annihilation whereas the ~200 ps component, independent of excitation intensity, is an intrinsic process associated with quenching of Chl *a* excited states.

This quenching component, identified also in the IsiA complexes,[18,19,28] is significantly slower than energy transfer between Pcb and PS I complexes that occurs on a time scale of a few picoseconds. Thus, unless the PS I is completely closed, the energy transfer from Pcb to PS I will always dominate, preventing loss of excitation via the 200 ps channel. On the basis of our data, we cannot speculate about the quenching mechanism, but similarity of our data with those reported[28] for IsiA points to the same process. While in IsiA the most likely quencher was the carotenoid echinenone,[28] in Pcb it could be zeaxanthin whose S_1 energy should be, based on comparison with LHCII,[42] below the Q_y state of Chl *a*.

It should be noted that no quenching was observed in another member of this protein family, CP43, whose lowest Chl *a* molecules have a lifetime of ~3 ns.[24] Thus, the intricate

equilibrium between energy transfer and quenching processes is specific to the IsiA and Pcb proteins that form rings surrounding the PS I trimers. These systems evolved a specific light-harvesting strategy that allows them to survive at very-low-light conditions due to enhanced light-harvesting capacity achieved by the antenna rings, but simultaneously enables to cope with high-light conditions, because the carotenoids in the Pcb and IsiA act as efficient quenchers upon excess light.

Conclusions

The results presented here show that the Pcb complexes of green oxyphotobacteria have similar function as the IsiA complexes of cyanobacteria. Pcb complexes enhance light-harvesting capacity by doubling the antenna size, but simultaneously act as effective quenchers that protects against excess light. The low efficiency (~25%) of the carotenoid−Chl energy transfer shows that the primary function of carotenoids in Pcb is not light harvesting. Interestingly, contrary to all light-harvesting systems studied so far, a substantial part of energy is transferred from the hot S_1 state of carotenoids. The key light-harvesting pigments in Pcb proteins are chlorophylls that, upon low-light conditions, greatly enhance the light-harvesting capacity of the Pcb−PS I system. Contrary to the IsiA complexes, Pcb further increases their cross section by utilizing Chl *b*, which transfers energy to Chl *a* with nearly 100% efficiency, and whose Soret band absorbs close to maximum of the solar irradiance curve.[43] Thus, upon low-light conditions, which is the natural habitat of these organisms, the Pcb−PS I system is able to capture as many photons as possible and transfer them efficiently to the reaction center. If, however, the organism is exposed to a harmful excess light, the carotenoids in Pcb proteins serve as efficient quenchers of excited chlorophyll molecules. Although the exact mechanism of the quenching remains unresolved, it is obvious that the Pcb−PS I antenna system is tuned to cope with both low-light and high-light conditions without limiting the light-harvesting capacity or risking damage to photosynthetic apparatus by excess light.

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Supporting Information Available: Comparison of excited-state dynamics of Pcb−PS I and PSI complexes, global fitting in carotenoid and Chl *a* spectral regions, and kinetics measured with different excitation intensities. This material is available free of charge via the Internet at http://pubs.acs.org.

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