Femtosecond Fluorescence Upconversion Studies of Light Harvesting by β-Carotene in Oxygenic Photosynthetic Core Proteins

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Received: July 13, 2004; In Final Form: September 14, 2004

Energy transfer from β-carotene to chlorophyll in the photosystem (PS) I core complex and the CP43, CP47, and reaction center (RC) proteins of PSII was studied by the femtosecond fluorescence upconversion technique. The carotenoid S_2 lifetimes/transfer efficiencies, the latter obtained by comparison with β-carotene in solution, are ∼97 fs/33% (CP43), ∼100 fs/31% (CP47), ∼106 fs/26% (PSII−RC), and ∼62 fs/57% (PSI). By combining previous steady-state fluorescence excitation measurements with our ultrafast results on the carotenoid S_2 lifetime and chlorophyll rise kinetics, we conclude that the β-carotene S_1 state is insignificant for light harvesting in the PSII core proteins. Measurement of the steady-state fluorescence excitation spectrum of PSI at 77 K in this work yielded a β-carotene to chlorophyll energy transfer efficiency of 62 ± 3%, suggesting dominant transfer from the carotenoid S_2 state. Interestingly, while both PSI and PSII almost exclusively utilize the carotenoid S_2 state for light harvesting, we find that this pathway is nearly two times more efficient in PSI.

Introduction

The two photosystems found in oxygenic photosynthetic organisms generally work in series to perform the light reactions of photosynthesis by carrying out complementary, yet distinct functions. Photosystem (PS) II coordinates the movement of two electrons from water to plastoquinone. The electrons are then transferred to either plastocyanin (plants) or cyctochrome c_6 (some cyanobacteria) where they are utilized by PSI to reduce $NADP^+$ to NADPH via a ferredoxin-like compound. The machinery of each PS has been optimized for its respective reaction, resulting in a number of structural and spectroscopic differences between the two supercomplexes and their corresponding proteins. One specific example is the variation in the amount of carotenoid (Car) light harvesting. The Car to chlorophyll (Chl) energy transfer efficiency in the core protein complexes of each PS measured previously by steady-state fluorescence excitation spectroscopy at 77 K was ∼30% for the PSII−Reaction Center (RC) from spinach,[1] ∼35% for CP43 and CP47 from spinach,[2] and ∼85% in PSI trimers from *Synechocystis* PCC 6803.[3] We attempt to obtain a more detailed understanding of these differences by utilizing the femtosecond fluorescence upconversion technique to quantify the involvement of the different Car excited states in the light harvesting process.

The PSII core is comprised of four pigment binding proteins, CP43, CP47, D1, and D2, all of which have the ability to harvest light. CP43 and CP47 (encoded by the psbD and psbC genes, respectively) are relatively similar proteins located adjacent to the PSII reaction center (RC).[4] Both have a dual light harvesting role, absorbing energy directly and transferring energy absorbed by the peripheral light harvesting antenna, which includes light harvesting complex II (LHCII), CP24, CP26, and CP29, to the RC. Structurally, CP43 and CP47 have six transmembrane helices,[5] a large luminal loop consisting of approximately 150 amino acids between helices V and VI, their N- and C-termini located on the stromal side of the thylakoid membrane, and many of the same histidine residues which coordinate the binding of Chl *a* via the central Mg atom.[4] Two distinct differences between the CP43 and CP47 proteins are that CP47 has a stronger association with the PSII-RC than CP43[6−8] and CP47 binds the single Chl *a* pigment, believed to absorb at 690 nm, that is responsible for the long wavelength fluorescence observed at 695 nm (F695).[9−11] The D1 and D2 subunits (products of the psbA and psbB genes, respectively) along with cytochrome b559 (encoded by psbE and psbF), PsbI, and PsbW comprise the PSII-RC complex.[12−14] D1 and D2 bind the reaction center pigments, 4−6 Chls *a*, 2 pheophytins, and 2 quinones, which are responsible for charge separation.[5,12,15,16]

The PSI core complex of *Synechococcus elongatus* is organized into trimers with each monomer composed of the PsaA to PsaF and PsaI to PsaM subunits.[17,18] While it is not possible to isolate only the subunits that bind the PSI-RC pigments from the additional Chl binding subunits, as is the case in PSII, the majority of the Chls in PSI form an antenna-like structure that, like CP43 and CP47, harvests energy for charge separation.[19] One distinct structural difference between the PSI and PSII core complexes is that the former binds sets of pigments that have excited-state energies below the energy of the PSI-RC special pair, P700, known as "red pigments". *Synechococcus elongatus* has two sets of these red pigments which at 4 K absorb at 708 and 719 nm.[20,21] The exact role of these pigments is currently not known; however, they generate a significantly broader spectral absorption range for PSI with respect to other pigment protein complexes and may be important for maximizing its light harvesting efficiency.[22]

The variation in Car to Chl energy transfer efficiency seems quite remarkable when compared with the fact that all of the complexes use β-carotene as their only light harvesting Car and

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10.1021/jp046893p CCC: $27.50
Published on Web 11/11/2004

Chl *a* as its energy acceptor. Both CP43 and CP47 bind approximately 2 β-carotene molecules and 15 Chls *a*.[5,16,23,24] The Cars in CP47 are believed to be all-trans;[5,16,23−26] however, no complementary information is available for CP43. The PSII-RC binds, in addition to the pigments necessary for primary charge separation, 2 β-carotenes which have 0−0 transitions that absorb at 490 and 506 nm and lie out and in the membrane plane, respectively. It has been suggested that the two Cars are excitonically coupled.[27,28] It is generally accepted that at least one of the two Cars adopts the all-trans conformation. The crystal structure of PSI revealed 96 Chls *a* and 22 β-carotenes per monomer: 16 are believed to be all-trans and 5 have one or two cis bonds.[29] The structure of the final PSI β-carotene was not modeled.

While steady state fluorescence spectroscopy measures the overall Car to Chl energy transfer efficiency, ultrafast spectroscopic studies enable quantification of the efficiency of the energy transfer pathways in the complexes. Two Car excited electronic states are generally considered to be involved in energy transfer. One state is the optically allowed S_2 ($^1B_u^+$) state, which absorbs light in the blue-green (∼420−510 nm) portion of the spectrum, a region where Chl has little to no absorption. The other state, S_1 ($^1A_g^-$), is optically dark. Under natural sunlight conditions, the S_1 state is populated by internal conversion from the S_2 state on a time scale of 100−200 fs.[30] Recently, spectroscopic evidence suggesting two additional Car excited states, S* and $^1B_u^-$, has been obtained, and these states are proposed as donors to bacterio(Chl).[31,32] While a number of studies on different light harvesting complexes (LHCs) agree that the dominant portion of the energy transfer to Chls via Cars proceeds from the Car S_2 state,[33−37] a role for the Car S_1 state in light harvesting has also been observed and is generally utilized in proteins which have an overall Car to Chl energy transfer efficiency in excess of ∼60%.[33−35,38] The first and second singlet excited states of Chl *a*, Q_y and Q_x, respectively, accept energy from β-carotene.

Previous transient absorption (TA) studies addressed the ratio of Car S_2/Car S_1 to Chl energy transfer efficiency in the PSI[39] and PSII[2] core light harvesting complexes. The Car S_2 lifetimes obtained upon excitation at 510 nm and fitted by means of a global analysis of the decay of the Car S_2 state and rises of the Car S_1/Chl population were 70 fs for CP43, 80 fs for both CP47 and the PSII-RC, and 60 fs for PSI, all with an error of ±20 fs. A previous fluorescence upconversion experiment by Kennis et al.[38] on PSI measured a value of ∼105 fs for Car S_2 lifetime upon excitation at 510 nm and detection at 580 nm. While the Car S_2 lifetime measured by both methods should, in principle, give the same result, differences in time resolution and technique, especially when measuring lifetimes on the order of 100 fs, have previously hampered extraction of the Car S_2 lifetime. One advantage of using the femtosecond fluorescence upconversion technique for these experiments is that the signal directly measures Car S_2 fluorescence without interference from additional overlapping transitions, coherent coupling, and cross phase modulation effects that can complicate TA data. Upconversion measurements on isolated LHCII trimers showed that with high time resolution, sub-100 fs lifetimes can be reliably extracted from fluorescence upconversion data.[40] The method is particularly useful in the study of complexes such as PSI where a ∼45 fs lifetime difference corresponds to a significant difference in the energy transfer efficiency of the S_2 state.

In this work, we employ the femtosecond fluorescence upconversion technique with ∼100 fs time resolution (on the same order as the previous TA measurements, but significantly better than the ∼300 fs resolution of the previous upconversion measurement on PSI) to gain a more in-depth understanding of the energy transfer from β-carotene to Chl *a* in the core PSII antenna proteins, CP43 and CP47, and in the multiprotein subunits that make up the PSI and PSII RCs. We have also measured the 77 K fluorescence excitation spectrum of PSI in order to clarify the role of the S_1 state in energy transfer. Collectively, these findings are significant for understanding how the core complexes utilize the same raw materials, i.e., pigments, for different ends.

Materials and Methods

Sample Isolation and Preparation. CP43, CP47, and PSII-RC complexes were isolated from spinach. First, *n*-dodecyl β,D-maltoside (β-DM) was used to isolate CP43 and CP47/PSII-RC complexes.[7,41] The CP47/PSII-RC complexes were further purified to CP47[42] and the PSII-RC with short Triton X-100 treatment.[1,15] PSI trimers were isolated from the cyanobacterium *Synechococcus elongates*.[43] After isolation, all of the samples were frozen in liquid nitrogen and stored at −80 °C until use.

For the measurement of the steady-state fluorescence excitation spectrum, PSI trimers of *Synechococcus elongatus* were dissolved in buffer (0.05% (w/v) β-DM, 20 mM $CaCl_2$, 20 mM $MgCl_2$, 10 mM MES, pH 6.5) and mixed with glycerol (66% v/v) to a final OD in the Chl Q_y band of 0.03/cm. The sample was contained in a 1 cm plastic cuvette, placed in a liquid nitrogen cryostat (Oxford) and cooled to 77 K.

For the fluorescence upconversion experiments, the samples were thawed immediately prior to measurement with the exception of some Chl fluorescence upconversion measurements on CP43, CP47, and PSI which were carried out on the following day after storage at 4 °C overnight. Buffers for CP43 (0.03% (w/v) β-DM, 20 mM NaCl, and 20 mM Hepes, pH 7.5), CP47 (0.03% (w/v) β-DM, 20 mM NaCl, and 20 mM BisTris, pH 6.5), and PSI (0.05% (w/v) β-DM, 20 mM $CaCl_2$, 20 mM $MgCl_2$, 10 mM MES, pH 6.5 and, added prior to measurement, 10 mM sodium ascorbate and 10 μM phenazine methosulfate (PMS)) were used to dilute the samples to the desired OD of ∼0.3/mm at 490 nm. The sodium ascorbate and PMS generally work to keep the PSI reaction center open, which is not possible in this experiment due to the repetition rate of the laser. We add them here, however, because they have been previously observed to decrease photodamage to the sample.[38] For the PSII-RC complex, no dilution of the samples was necessary. An oxygen scavenging system (20 mM glucose, 0.038 mg/mL catalase (Sigma C-100) and 0.1 mg/mL glucose oxidase (Sigma G-6125))[44] was added to the PSII-RC samples to maintain anaerobic conditions. The samples were continually flowed and cooled (6−8 °C) during the measurements. Absorption spectra taken before and after each measurement (Shimadzu UV-1601) showed no noticeable differences from each other and from the original absorption spectra measured immediately after isolation of the protein.

β-Carotene (Sigma 22040, ≥97%) was used without further purification and was dissolved in *n*-hexane (OmniSolv, 98.2%), toluene (Aldrich, 99.5%), and cyclohexane (OmniSolv, 99.97%). The β-carotene upconversion measurements were preformed at room temperature (22 °C) on samples with an OD of ∼0.3/mm at 490 nm.

Steady-State Fluorescence Excitation Spectrum. The fluorescence excitation spectrum of PSI was measured by using a commercial fluorometer (Jobin-Yvon Fluorolog 3) with right-angle detection. The excitation bandwidth was 1 nm and the fluorescence was detected at 725 nm with a 5 nm band-pass.

Femtosecond Fluorescence Upconversion. Femtosecond fluorescence upconversion experiments have been described in detail previously.[45−47] Briefly, a Ti:sapphire oscillator (Coherent MIRA Seed) was used to seed a regenerative amplifier (Coherent RegA 9050) with an external stretcher/compressor that pumped an OPA, optical parametric amplifier (Coherent 9450). The OPA produced excitation pulses centered at 490 nm with a pulse energy of ∼5 nJ, a repetition rate of 250 kHz, and a temporal fwhm of ∼50 fs. A portion (30%) of the 800 nm compressor output was used as the upconversion gate beam. The sample, contained in a custom, 1 mm path length flow cell (Starna Inc.), was placed at one focus of the elliptical mirror. The spontaneous emission from the sample was collected by the mirror and upconverted with the gate beam at the mirror's second focus in a BBO Type I crystal (Inrad, thickness = 0.25 mm, $\theta = 28.7°$). A lens was used to collect the upconverted signal, which was directed into a double grating monochromator (Spex 1680) and detected by a photomultiplier tube using gated photon counting (Stanford Research Systems SRS400).

For measurements of the Car S_2 fluorescence, the polarization of the pump beam was parallel with respect to the gate beam. Chl fluorescence measurements were preformed with the respective polarizations of the pump and gate beam at magic angle (54.7°). Polarizations were set with an achromatic polarizer placed in the pump beam (CVI ACWP-400-700-10-2).

The IRF is generally well described by a Gaussian function fitted to the pure Raman scattering signal of the solvent[48] in exactly the same experimental configuration used to measure the Chl/Car S_2 fluorescence. The IRF ranged from ∼100 (buffer) to ∼150 fs (*n*-hexane/toluene/cyclohexane).

Data Analysis: Fluorescence Upconversion. Single and biexponential fits of the Car S_2 lifetime with a Gaussian IRF were carried out by two nonlinear least-squares fitting programs, Foppefit and Spectra (S. Savikhin Software, Ames, IA). The Spectra program was also used to fit the Chl fluorescence data.

Results

Steady-State Absorption and Fluorescence. The absorption spectra of β-carotene in *n*-hexane (index of refraction, *n*, = 1.37), cyclohexane ($n = 1.43$), and toluene ($n = 1.50$) are shown in Figure 1A. The transition observed is the Car S_2 state and the corresponding solvent dependent shift is well-known to arise from the almost linear dependence of the transition on the index of refraction portion of the polarizibilty, $R(n)$.[30] The full absorption spectra of the individual PSII core proteins, CP47, CP43 and the PSII-RC, and PSI are shown in Figure 1B. Noticeable differences between these spectra occur in the region from ∼470−510 nm, reflecting variation in the Chl *a*/β-carotene ratio in each of these proteins, and in the region from ∼700−750 nm due to presence of "red" Chls specifically in PSI.

The fluorescence excitation spectrum, detected at 725 nm, and the corresponding 1-transmission (1-T) spectrum for PSI trimers of *Synechococcus elongatus* were measured at 77 K and normalized to the Chl Soret region, Figure 2. By comparison of the two spectra, we find the overall energy transfer efficiency from β-carotene to Chl in these complexes is 62 ± 3%.

Time-Resolved Fluorescence Measurements. β-Carotene S_2 Lifetime in Solution and in the Core Protein Complexes. Femtosecond fluorescence decay traces for β-carotene in solution detected at 560 nm, and their corresponding single-exponential fits, upon excitation at 490 nm are shown in Figure 3A. Iterative reconvolution fitting of the β-carotene fluorescence decays in toluene, cyclohexane, and *n*-hexane with a Gaussian IRF gave single-exponential lifetimes of 144, 166, and 172 fs (all with an error of ±10 fs), respectively. These values are within the experimental error of those obtained previously.[30] The decay kinetics of each of the core Chl proteins, also excited at 490 nm and detected at 560 nm, and their corresponding single-exponential fits are shown in Figure 3B. The value of

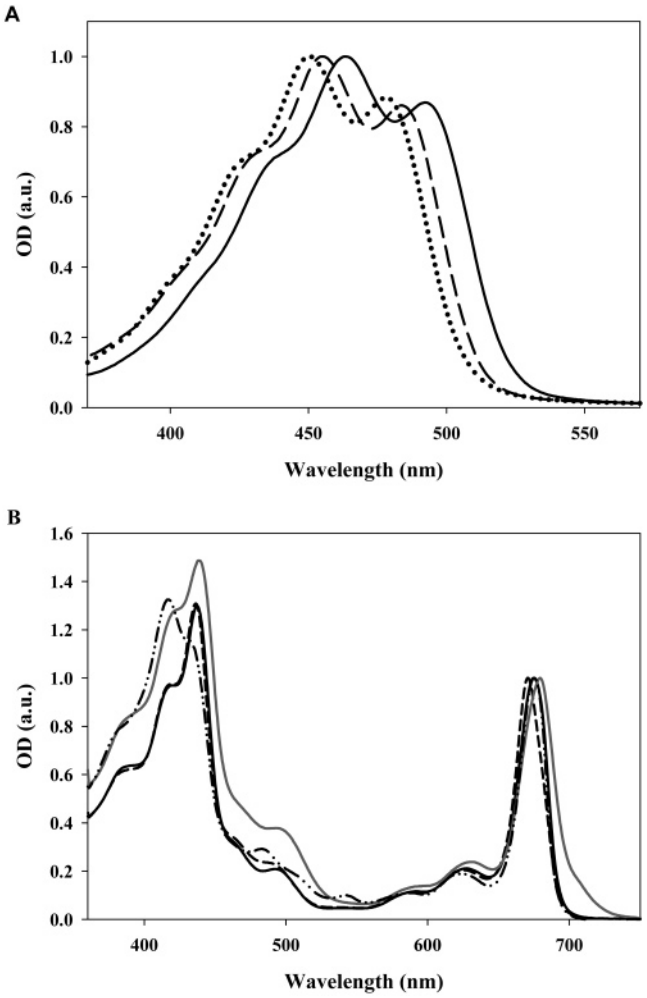

Figure 1. (A) Normalized absorption spectra of β-carotene in toluene (solid line), cyclohexane (long-dashed line), and *n*-hexane (dotted line). (B) Absorption spectra of Chl proteins: CP43 (dashed line), CP47 (solid line), PSII-RC (dot dot dashed line), and PSI (gray) normalized with respect to the Chl Q_y absorption maximum.

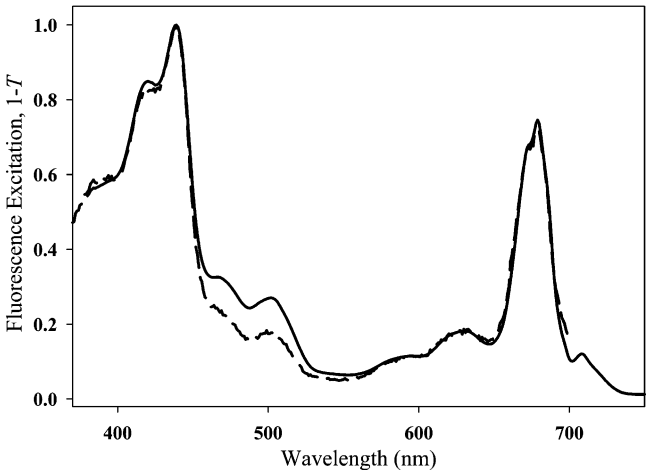

Figure 2. Normalized fluorescence excitation (solid line) and 1-T (dashed line) spectra for PSI trimers at 77 K.

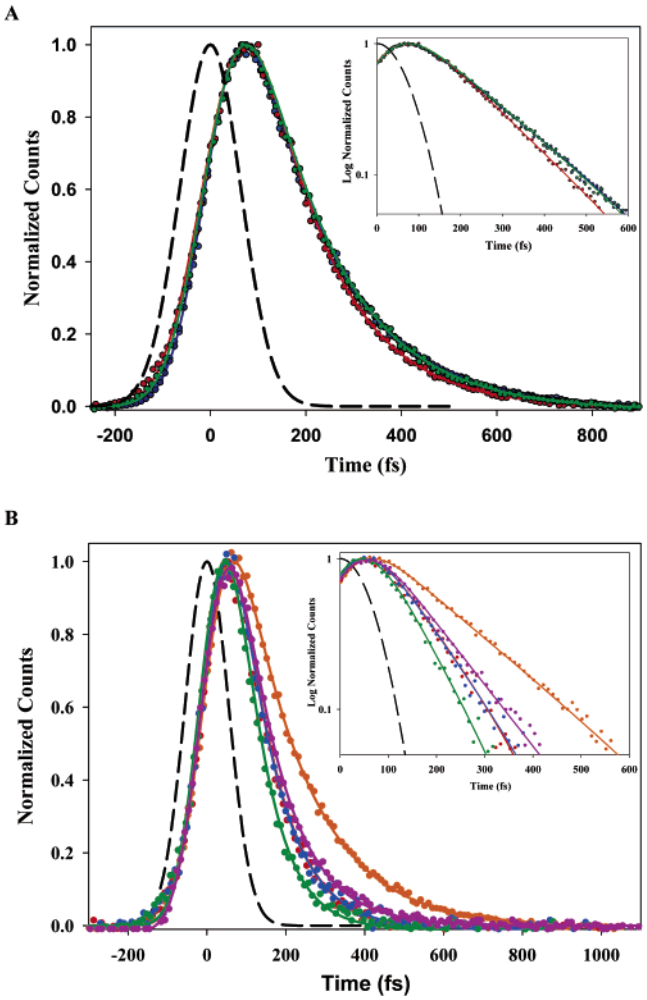

Figure 3. (A) Normalized experimental upconversion traces of β-carotene (circles) and their corresponding single-exponential fits (solid lines) in toluene (red), cyclohexane (green), and *n*-hexane (blue). The inset shows the same data with the counts on a logarithmic scale. A Gaussian IRF of 150 fs is also shown (dashed line). (B) Normalized experimental upconversion traces of chlorophyll proteins (circles) and their corresponding single-exponential fits (solid lines): PSI (green), CP47 (red), CP43 (blue), and PSII-RC (purple). Experimental data for β-carotene in *n*-hexane (orange circles) are also shown with a single-exponential fit (orange line). The inset shows the same data with the counts on a logarithmic scale. A Gaussian IRF of 130 fs is also shown (dashed line).

TABLE 1: Fluorescence Lifetimes for the β-Carotene S_2 State and Corresponding Energy Transfer Efficiencies (protein complexes only) upon Excitation at 490 nm and Detection at 560 nm[a]

sample	τ_{S2} (fs)	φ_{S2} (%)
toluene	144	
cyclohexane	166	
n-hexane	172	
CP43	97	33
CP47	100	31
PSII-RC	106	26
PSI	62	57

[a] Lifetimes are within ±10 fs. Energy transfer efficiencies are within ±7%.

the S_2 lifetime obtained from these fits was 100 (CP47), 97 (CP43), 106 (PSII-RC), and 62 fs (PSI) each with an error of ±10 fs (Table 1). PSI was the only sample that showed some improvement upon fitting the decay with a double exponential fit with components of 31 (96%) and 124 fs (4%). At first it may seem logical to assign the former component as the average S_2 lifetime of the Cars that transfer energy to Chl and the latter to arise from either disconnected Cars in the sample or from excited Cars associated with triplet Chl molecules.[49] However, a previous upconversion study on spirilloxanthin in solution, the Car with the shortest reported S_2 lifetime (69 ± 10 fs), also showed improvement in the fit quality when a double-exponential as opposed to single-exponential fit was used. This clearly cannot be related to differences in energy transfer efficiency.[40] Upon careful inspection, the deviation from a single-exponential fit in spirilloxanthin was shown to be an experimental artifact and an additional exponential was not necessary to adequately characterize the data. Combining these findings on spirilloxanthin with the fact that a major Car S_2 to Chl energy transfer component in PSI of ~31 fs would yield a larger energy transfer efficiency from the S_2 state than is attained for the whole complex by steady-state fluorescence measurements leads us to conclude that even though there is probably heterogeneity between the transfer efficiency of the 22 β-carotenes in PSI, the single-exponential fit value of 62 ± 10 fs best represents the average Car S_2 lifetime for PSI.

Chl Fluorescence Rise Kinetics in the Core Complexes. Chl fluorescence traces for each of the core complexes were measured upon excitation at 490 nm. The Chl kinetics detected at 666 and 678 nm and normalized at the maximum for CP47 and their corresponding fits are shown in Figure 4A. For comparison, the kinetics detected at 678 nm for CP43 and CP47 are shown in Figure 4B. The values of the fits for the kinetic traces measured for CP47 and CP43 are summarized in Table 2. All the traces show a major rise component on the order of ~100 fs. Only the two longest wavelength traces measured for both CP43 and CP47, 678 and 685 nm, show a small, but clear improvement when a biexponential function is used to fit the data as opposed to a single exponential. The second exponent in these four traces has a lifetime of ~300 fs and amplitude of ~30%. The fluorescence from the PSII-RC complexes at 670 and 685 nm shows single-exponential rises on the order of ~110 fs, Figure 5. The PSI Chl kinetics upon excitation at 490 nm and detection at both 668 and 692 nm are shown in Figure 6. Previous upconversion measurements on PSI trimers excited at 400 nm,[38] a wavelength where Car absorption is negligible, allowed for the extraction of the intraband Chl energy transfer time scales in this system. By fixing these components in our analysis, a time constant of ~65 fs was extracted upon detection at 668 and 692 nm with amplitudes of ~100% and ~33%, respectively.

Lifetime and Efficiency of Energy Transfer from the Car S_2 State. For the PSII core protein complexes, a rise component in the Chl *a* fluorescence was observed, which accounted for either the full rising amplitude or a major component of it, that matched the lifetime of the decay of the Car S_2 state. The findings indicate that the shortening of the lifetime measured for β-carotene in the complexes with respect to solution was due to energy transfer. From the measured values of the Car S_2 lifetime, τ_{S2}, we can estimate the lifetime and efficiency of energy transfer from the β-carotene S_2 state to Chl *a* in each of the core complexes. The rate of energy transfer, k_{ET}, and the lifetime of energy transfer, τ_{ET} ($=k_{ET}^{-1}$), can be calculated from the relation $k_{ET} = k_{S2} - k_{IC}$, where k_{S2} ($=\tau_{S2}^{-1}$) is the rate of decay of the Car S_2 state in the protein complex and k_{IC} ($=\tau_{IC}^{-1}$) is the internal conversion (IC) rate of the β-carotene S_2 state in the absence of energy transfer. The efficiency of energy transfer from the β-carotene S_2 state to Chl *a*, φ_{S2}, is given by the expression $\varphi_{S2} = k_{ET}/(k_{ET} + k_{IC})$.

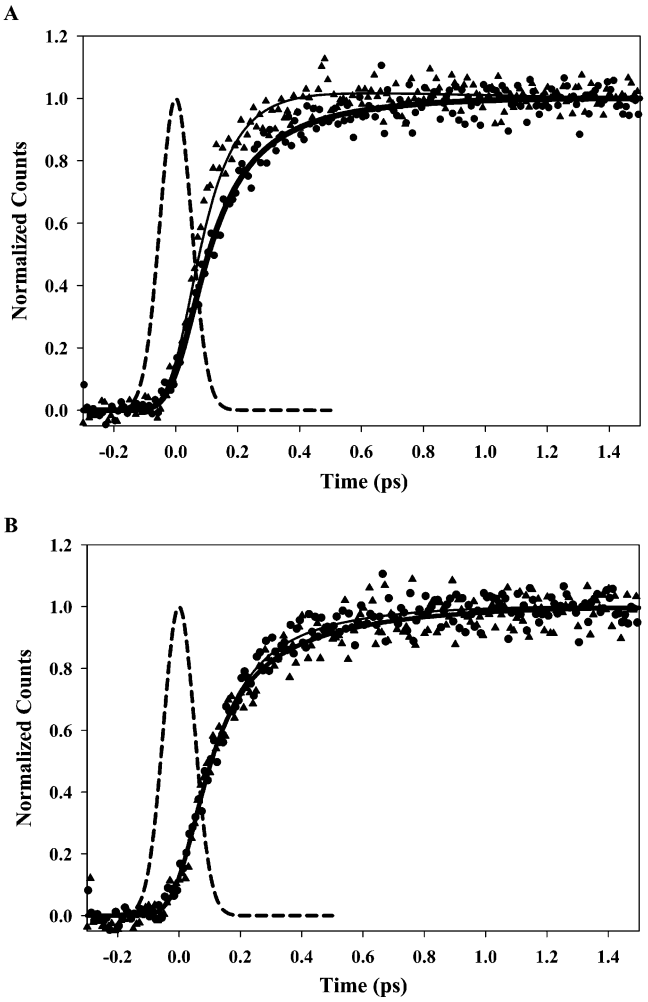

Figure 4. (A) CP47 Chl fluorescence normalized at 1.5 ps upon excitation at 490 nm and detection at 666 nm [data (triangles) and fit (thin line)] and 678 nm [data (circles) and fit (thick line)]. A Gaussian IRF of 120 fs is also shown (dashed line). (B) Chl fluorescence normalized at 1.5 ps upon excitation at 490 nm and detection at 678 nm for CP47 [data (circles) and fit (thin line)] and CP43 [data (triangles) and fit (thick line)]. A Gaussian IRF of 120 fs is also shown (dashed line).

TABLE 2: Chl Fluorescence Kinetics for CP47 and CP43[a]

sample	detection λ (nm)	τ_1 (fs)	A_1 (%)	τ_2 (fs)	A_2 (%) CP47
CP47	666	100	−100		
	671	112	−100		
	678	110	−74	360	−26
	685	100	−65	300	−36
CP43	671	118	−100		
	678	97	−65	344	−35
	685	114	−70	277	−30

[a] Only the rise times are shown because the sample decays on a time scale that cannot be accurately determined due to the collection window.

To determine the rate and efficiency of energy transfer from the Car S_2 state, we must be able to estimate τ_{IC}. Two previous studies concluded that the index of refraction experienced by β-carotene in the CP47 protein environment is ~1.5,[50,51] the same value as for the solvent toluene. Since the Car S_2 state has a much larger transition dipole moment than the Car S_1 state (which is nearly zero), for all intents and purposes, only the energy shift of the former state depends on the index of refraction portion of the polarizability.[30,52] Therefore, the energy

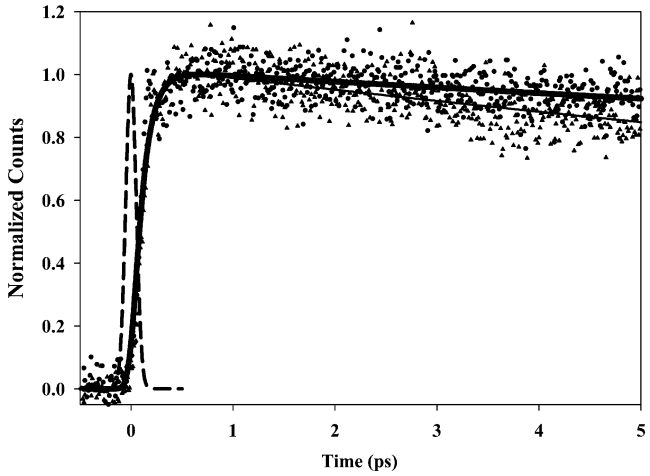

Figure 5. PSII Reaction center Chl fluorescence normalized at the absorption maximum upon excitation at 490 nm and detection at 670 nm [data (circles) and fit (thick line)] and 685 nm [data (triangles) and fit (thin line)]. A Gaussian IRF of 120 fs is also shown (dashed line).

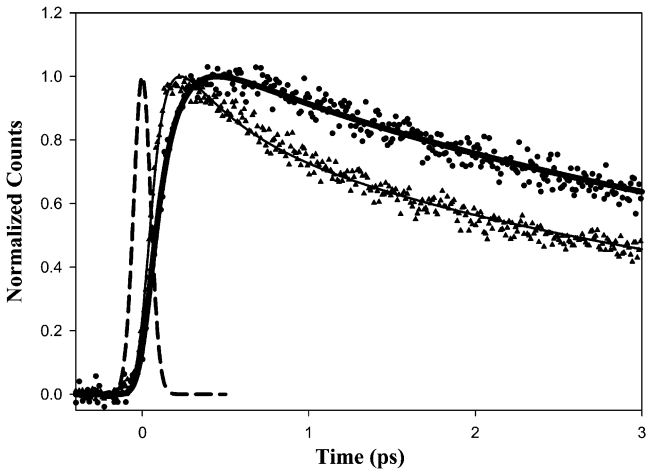

Figure 6. PSI Chl fluorescence normalized at the absorption maximum upon excitation at 490 nm and detection at 668 nm [data (triangles) and fit (thin line)] and 692 nm [data (circles) and fit (thick line)]. A Gaussian IRF of 120 fs is also shown (dashed line).

difference between the β-carotene S_2 and S_1 states should be the same in CP47 and toluene, suggesting that the value obtained for the S_2 lifetime in toluene (144 fs) should correctly characterize τ_{IC} in CP47. Although the corresponding measurement of the index of refraction of CP43 has not been carried out, the strong similarities between CP43 and CP47, including the experimentally identical values for the Car S_2 lifetime and Chl rises, imply that it is reasonable to assume that both proteins can be characterized by the same τ_{IC} value. From this assumption, we find that for both CP43 and CP47 $\tau_{ET} = \sim 300$ fs and $\varphi_{S2} = \sim 32\%$. Since no data currently exist for the index of refraction experienced by the two β-carotenes in the PSII-RC complex, we assume that the value is similar to CP47 and find that $\tau_{ET} = \sim 400$ fs and $\varphi_{S2} = \sim 26\%$ (Table 2).

For PSI, the Chl fluorescence kinetics also have a rise component that matches the decay lifetime of the Car S_2 state although it appeared with only minor amplitude for the trace measured at 692 nm. Although no measurement of the index of refraction for PSI has been made, the ~90 Chls associated with the PSI-RC are somewhat structurally and functionally homologous to the Chls bound to CP47,[5,19,53] suggesting that the index of refraction for the two proteins is comparable. Under this assumption, we calculate that $\tau_{ET} = \sim 110$ fs and $\varphi_{S2} = \sim 57\%$.

Discussion

By comparing the previously measured overall Car to Chl energy transfer efficiency with the value extracted from our time-resolved fluorescence measurements, we find that in each of the PSII core complexes the Car S_2 energy transfer pathway accounts for almost all, if not all, of the β-carotene to Chl energy transfer. These results qualitatively agree with a previous TA study by de Weerd et al.,[2] which found no evidence for relaxed Car S_1 to Chl energy transfer and a minor, if any, component of transfer from vibrationally excited Car S_1 states. However, we measure lifetimes for the Car S_2 state in the PSII core complexes that are, on average, ~30 fs longer than those measured by de Weerd et al. Such deviations can be significant for calculations of the energy transfer time scales and efficiencies because τ_{IC} for the β-carotene S_2 state is ~100−200 fs.[30]

Our steady state and time-resolved measurements find that, like in the PSII core complexes, energy transfer from the optically allowed Car S_2 state to Chl in PSI can also account for almost all to all of the light harvesting process from β-carotene. In a recent study, the spectral evolution of optically dark states of β-carotene in *Synechococcus elongatus* trimers of PSI was directly measured by means of ultrafast transient absorption spectroscopy.[39] From the kinetic data it was estimated that the total light harvesting efficiency in PSI amounted to 70%, of which the S_1 channel contributed 10%. Previous fluorescence upconversion experiments on *Synechococcus elongatus* trimers of PSI concluded that the Car S_2 energy transfer efficiency was ~60% by means of a target analysis of the Chl kinetics obtained upon excitation of β-carotene at 510 nm and exclusive excitation of Chl at 400 nm.[38] While this value is in good agreement with our current findings, we note that both the measured Car S_2 lifetime in the work of Kennis et al., 105 fs, and the extrapolated τ_{IC} for β-carotene, 250 fs, are significantly longer than the values obtained in this work.

The previous fluorescence upconversion data[38] also showed a rise component of the Chl fluorescence upon Car excitation that was not present upon direct Chl excitation. The analysis concluded that ~35% of the total energy was transferred via this pathway and was assigned to the S_1 state of β-carotene. While Kennis et al.[38] concluded that a total energy transfer efficiency of ~95% is reasonable because previous fluorescence excitation measurements on PSI *Synechocystis* PCC 6803 trimers show a total β-carotene to Chl energy transfer efficiency of ~85%, our corresponding steady state and time-resolved fluorescence results on *Synechococcus elongatus* trimers of PSI make it difficult to accept such a large involvement of the Car S_1 state in light harvesting.

While the PSI TA results of de Weerd et al.[39] and our current measurements show general agreement, the exact reason for the discrepancy between these two experiments and the previous fluorescence upconversion results[38] is unclear. The differences found between the previous upconversion and TA measurements for the time constant of Car S_1 transfer (1.2 and 3 ps, respectively) and relative contributions (35% and 10%, respectively) may arise from the interpretation of the global analysis results and simplifications in kinetic modeling. For instance, the global analysis results of de Weerd et al. showed a pronounced 300 fs component that was assigned to a "hot" S_1 state. Upon vibrational cooling in 300 fs, a significant loss of total S_1 absorption was observed, which indicates that energy transfer from "hot" S_1 states to Chl may take place. Although this possibility was considered by de Weerd et al.,[39] it was not taken into account in the quantitative assessment of energy transfer pathways. In the target analysis of Kennis et al.,[38] only a single energy transfer time constant from S_1 was taken into account (1.2 ps), which may represent some weighted average of the 300 fs and 3 ps time constants found by de Weerd et al. In addition, the limited number of upconversion traces acquired in the work of Kennis et al. and the time resolution of the experiment may have prevented a complete disentanglement of the complicated Chl intraband dynamics from the β-carotene to Chl energy transfer.

We find that in both the PSI and PSII core complexes measured in this work, β-carotene to Chl energy transfer occurs dominantly from the S_2 state. If the index of refraction of the protein is correctly represented by β-carotene in toluene, as we assumed, then the S_1 state transfer pathway accounts for only a few percent, at most, of the overall energy transfer in all complexes. Furthermore, the results indicate that, for all intents and purposes, all of the energy transfer from β-carotene to Chl in the core complexes can be accounted for by transfer from only the Car S_2 and S_1 states. These findings raise doubt about the need to account for transfer via Car states such as $^1B_u^-$.

Interestingly, while energy transfer from the Car S_2 state is the dominant pathway used by each of the core complexes studied, the energy transfer efficiency of this state in PSI is approximately twice that of the PSII complexes. While the physical reason for these differences cannot be elucidated by the methods utilized here, it is unlikely that significant disparity exists in the strength of electronic coupling between Cars and Chls in PSI and PSII, since β-carotene's photoprotective function in PSII of triplet-state uptake requires van der Waals contact between Chl and Car.[54] Rather, the enhanced light harvesting efficiency of PSI likely arises from differences in the coordination of β-carotene by Chl molecules: in PSII, β-carotene is in close contact with only one or perhaps two Chl(s) *a*.[5] In PSI, almost every β-carotene is closely associated with multiple Chl *a* molecules,[29] which may each act as energy acceptors. Since 5 cis Cars have been assigned in PSI,[29] it is also possible that differences in the conformation of the β-carotenes affect energy transfer efficiency to Chl. Photophysical studies of cis β-carotene would help to clarify this issue.

Concluding Remarks

In this study, we present measurements of the Car S_2 lifetimes in the core complexes of PSII and PSI with the highest time resolution to date and establish that the lifetime shortening is due to Car to Chl energy transfer. The results show that while energy flows mainly via the Car S_2 state in both the PSI and PSII core proteins, the S_2 energy transfer efficiency is significantly higher for PSI. Since PSI trimers from *Synechococcus elongatus* and *Synechocystis* PCC 6803 show noticeably different overall Car to Chl energy transfer efficiencies (62% vs 85%[3]), studies which investigate the energy transfer pathways in the later complex may provide additional insight into whether the S_2 state can transfer even more efficiently or if some transfer proceeds via the S_1 state. Although none of the core complexes studied in this work efficiently utilize the Car S_1 state, we note that the major light harvesting antenna complex, LHCII, which has Chl *a* as the only pigment in common with the core complexes, has ~20% overall transfer efficiency proceeding via this state.[33,40,55] An in-depth understanding of these differences within the core complexes and between the core complexes and the antenna could greatly enhance our ability to develop highly optimized artificial photosynthetic systems.

Acknowledgment. This work was supported by the Director, Office of Science, Office of Basic Energy Sciences, Chemical

Sciences Division, of the U.S. Department of Energy under contract no. DE-AC03-76SF00098. The authors thank Sandrine d'Haene and Jan Dekker from the Vrije Universiteit Amsterdam for the gift of the PSII samples and Eberhard Schlodder from the Technische Universität Berlin for providing the PSI trimers from *Synechococcus elongatus*. We also thank Donatas Zigmantas and Harsha M. Vaswani for comments on the manuscript. John T. M. Kennis was supported by the Human Frontier Science Program Organization via a short-term fellowship.

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