Direct observation of ultrafast energy transfer in PSI core antenna

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A femtosecond fluorescence upconversion apparatus was used to measure the fluorescence decay in the PSI core antenna of a PSI-only mutant of *Chlamydomonas reinhardtii*. The fluorescence depolarization measurements reveal a fast depolarization with a time constant in the range of 150–300 fs irrespective of the excitation and/or detection wavelengths. We associate this time with the energy transfer timescale. The isotropic fluorescence decay shows a decay component of ≈ 5 ps and is suggested to result from spectral equilibration within the antenna.

1. Introduction

The first steps in photosynthesis are light harvesting by specialized antenna pigment proteins followed by trapping of the excitation in a photosynthetic reaction center and subsequent electron transfer. The light harvesting process involves energy transfer between pigment molecules. Both energy transfer and the trapping process are highly efficient, with quantum yields of more than 90% [1,2]. The process of energy migration within the antenna system of both plants and photosynthetic bacteria has been discussed at a variety of levels, from phenomenological kinetic schemes through analytical kinetic schemes to microscopic molecular models [1–7]. Little structural information is yet available for antenna systems, although important progress has been made recently on the light harvesting complex II (LHC-II) [8]. In the case of photosystem I (PSI) of higher plants, the reaction center and a "core" antenna of 80–100 chlorophyll **a** (Chl **a**) molecules appear to be part of the same protein–pigment complex [1,9]. At present the spatial arrangement of the antenna molecules is unknown. In addition, the energies of the Chl **a** molecules are modified through inter-chlorophyll interactions and through electrostatic and conformational perturbations generated by the protein [1,10,11].

An important elementary question concerns the nature and timescale of the initial energy transfer process in a system such as the core antenna of PSI. An early attempt to answer this question was made by Owens et al. [12], who studied the fluorescence decay times of PSI particles in which the average antenna size was varied by detergent treatment. By using the regular lattice model, an average single step transfer time (i.e. the inverse of the pairwise rate divided by the coordination number) of 0.22 ± 0.04 ps was obtained. In addition to the crudeness of the theoretical model, this result can also be criticized because the antenna size distribution obtained by Owens et al. [12] was likely inhomogeneous [13]. Struve and co-workers have observed that the pump–probe depolarization time in Chl **a** antenna photobleaching transients between 660 and 680 nm in native PSI particles occurs on a 5–13 ps timescale. They have discussed this result in terms of the excitation transfer between clusters of chromophores rather than between individual chromophores [14]. In very recent two-color experiments [15], Struve and coworkers have observed that the two-color anisotropy functions are dominated by an unresolvably fast decay within their time resolution, and they suggested

that mainstream energy transfer steps in the core antenna occur on a subpicosecond timescale. From hole-burning studies of the PSI core antenna complex at low temperature, Small and co-workers [16] initially proposed an energy transfer timescale in the range of 200–400 ps at 1.6 K, however in more recent studies Lyle and Small [17] have obtained energy transfer times of a few picoseconds at 4.2 K. They regard the very slow timescales obtained earlier as an artifact resulting from detergent damage to the sample.

We have recently developed a reflective optics fluorescence upconversion system with a time resolution of ≈ 40 fs. In this Letter, we present fluorescence decay and anisotropy data obtained from the core antenna of PSI particles aimed at reconciling the various observations described above.

2. Experimental

C. reinhardtii strain A4d, which contains PSI and its core antenna only, was grown in low light in tris-acetate phosphate (TAP) medium and PSI core particles were prepared as described previously [18]. Chl a/P700 ratios were determined as described in ref. [18] and ranged from 40–50 for these PSI particle preparations, which we refer to as PSI-40 particles.

Fluorescence decays were recorded using the fluorescence upconversion technique. The fluorescence upconversion setup and laser system are described in detail elsewhere [4,19,20]. In this work, the mixing crystal was a 400 μm thick $LiIO_3$ crystal. Pump pulse wavelengths centered at 630, 640, 650 or 656 nm were selected by 10 nm bandpass interference filter from a 100 kHz repetition rate continuum and amplified in a single pass dye jet (DCM) to 3–4 nJ per pulse, while the gate pulse was centered at 608 nm with 5–6 nJ per pulse. Typical pump-gate cross-correlation functions had a fwhm of 90–100 fs. Fluctuations in laser intensity were within a few percent, making normalization of the raw data unnecessary. The peak count rate was 10–80 per second depending on the wavelength and polarization detected.

Fluorescence decay curves were recorded with the polarization of the excitation pulse parallel, perpendicular or at the magic angle (54.7°) relative to the polarization of the gate pulse to obtain $I_{\parallel}(t)$, $I_{\perp}(t)$ and $I_{iso}(t)$. The anisotropy decay $r(t)$ and isotropic decay $K(t)$ are related to the deconvoluted fluorescence curves by

$$r(t) = [I_{\parallel}(t) - I_{\perp}(t)]/[I_{\parallel}(t) + 2I_{\perp}(t)] ,$$

$$K(t) = I_{iso}(t) = I_{\parallel}(t) + 2I_{\perp}(t) .$$

The measured parallel and perpendicular data were analyzed by simultaneous iterative reconvolution and nonlinear least-squares fitting of both data sets as described by Cross and Fleming [21]. The measured isotropic data were analyzed using the MINUIT package, and the fitted results, in turn, were used as the boundaries for anisotropy analysis. The instrument response function used in fitting was taken to have a similar shape to the measured cross-correlation function but to be 20–50 fs broader to take into account the group velocity mismatch between the fluorescence and the gate pulse. The amount of broadening varies with the detected fluorescence wavelength.

Care has been taken to assure accurate polarization measurements. No polarizer is used after the sample in order to maintain high time resolution and sensitivity. The S-polarized mixing beam and carefully aligned $LiIO_3$ crystal assure that only S-polarized fluorescence was upconverted. The solid angle of the fluorescence collection was reduced by masking the elliptical mirror in order to prevent scrambling of the S and P polarizations by the elliptical reflector. We verified that the initial anisotropy of cresyl violet in ethanol is 0.4 ± 0.02. Finally, the parallel and perpendicular fluorescence signals were obtained by rotating the polarization of the excitation beam, and the scans were taken alternatively for parallel and perpendicular polarizations to minimize the influence of any long term laser drift and possible sample degradation.

All measurements were carried out at room temperature. Absorption and fluorescence spectra, as well as the Chl a/P700 ratio, were used to monitor the sample. The optical density of the sample in a 1 mm pathlength measuring cell was 0.3–0.45 at 650 nm. The sample was stirred during the measurements using a filament attached to an electric toothbrush.

3. Results

We first studied Chl a in acetone solution, both as a test of the apparatus, and to check the photophysics of the chromophore of the PSI core antenna on ultrafast timescales. In dilute ether or acetone solution Chl a is a monomer [22]. Fig. 1 shows a fluorescence data set for Chl a in acetone solution with an optical density of 0.35 in a 1 mm pathlength at 650 nm. The excitation pulse was centered at 650 nm, and the cross-correlation function between the excitation pulse and the gate pulse had a width of 100 fs. Fluorescence was detected at 730 nm (in the region of the 0–1 vibronic band [23,24]) with a 4 nm bandwidth. Fig. 1a shows the fluorescence detected with parallel and perpendicular polarization with respect to the excitation polarization. Fig. 1b shows the fluorescence detected at the magic angle. An analysis of the data in fig. 1 using an instrument response function of 130 fs fwhm gives an initial anisotropy 0.39 ± 0.02. This anisotropy is almost constant up to 2 ps. These fluorescence curves clearly demonstrate that there is no ultrafast decay or depolarization component in Chl a solution and that the 0–1 transition moment has the same direction as that of the 0–0 transition. This is in agreement with studies of Myslinski and Koningstein on much longer time scales [24]. However, when the concentration is increased to an optical density of 1.0 in a 1 mm pathlength cell, the initial anisotropy drops to 0.3. This is consistent with earlier experimental results [22,24], showing that when the concentration of Chl a is increased, dimers or larger aggregates are formed. Whether the reduction in the initial anisotropy arises from unresolved dynamics or from delocalized states formed by strongly interacting molecules is not clear at present.

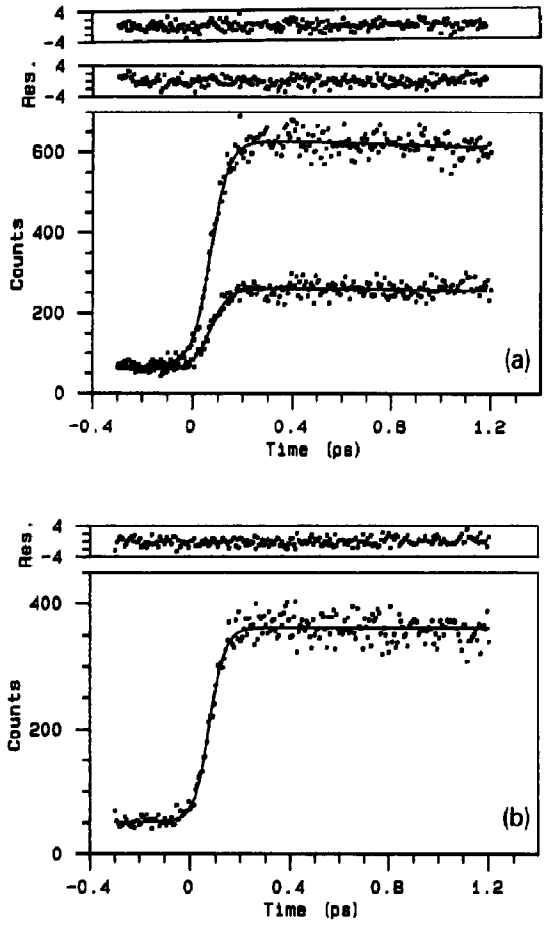

Fig. 1. Fluorescence data set for Chl a in acetone solution. (a) Parallel (top) and perpendicular (bottom) decay data (dots) and the fitted curves (solid lines). Residuals are shown above the data: top panel for parallel and bottom panel for perpendicular. (b) Isotropic decay (dots) and fit (solid line).

We now turn to the fluorescence measurements on PSI-40 particles. Fig. 2 shows the absorption and emission spectra of the PSI-40 particles at room temperature. The vertical arrows indicate the various excitation and detection wavelengths that were used in this study. Excitation is in the Q_y region of Chl a, and detection is in the red tail of the steady-state fluorescence spectrum; presumably the Chl a 0–1 vibronic band. Fig. 3 shows a typical data set recorded with 650 nm excitation and 730 nm emission detected with 4 nm bandwidth, along with fitted curves. A rapid decay component is clearly evident in the fluorescence anisotropy shown in fig. 3c. A fit including convolution with the instrument response function (120 fs fwhm) gives an initial value of the anisotropy of 0.34 ± 0.03, and an ultrafast anisotropic decay with a time constant of 180 ± 40 fs. The anisotropy has not decayed to zero by 2 ps. Longer scans reveal that $r(t)$ is in the range 0.1–0.2 up to 40 ps (fig. 4c). The magic angle curve (fig. 3b) does not show any ultrafast (i.e. <1 ps) decay or rising component.

Experiments carried out at all of the excitation and emission wavelengths indicated in fig. 2 produced

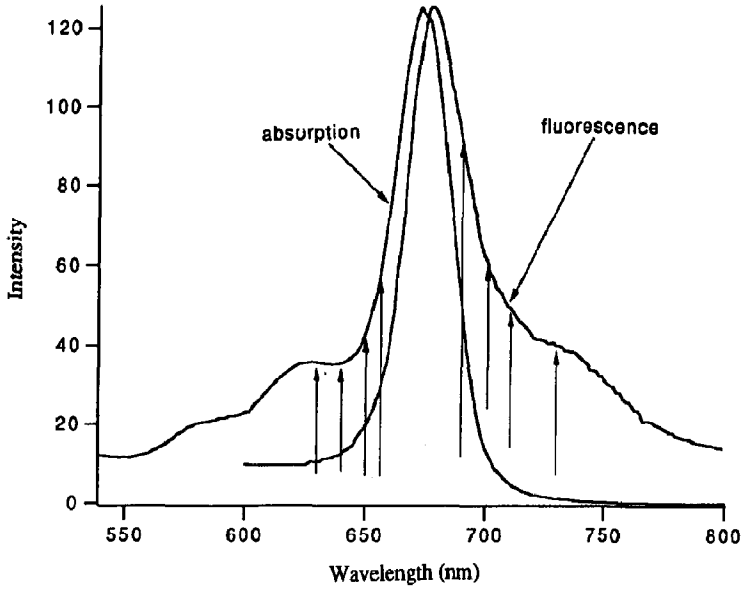

Fig. 2. Absorption and fluorescence spectra of PSI particles. Arrows indicate the center wavelengths of excitation and detection.

similar results, i.e. ultrafast anisotropy decays in the range 150–300 fs and an asymptotic value of 0.1–0.2 for $r(t)$ at 40 ps. With our present signal-to-noise ratio we are unable to accurately determine trends in $r(0)$ or the rapid decay time constants with excitation and/or detection wavelengths.

In our experiments we kept the excitation pulse energy at 3–4 nJ in order to minimize the possibility of singlet–singlet annihilation. We estimate the excitation intensity at the sample in our experiments is $\approx 1\times10^{13}$ photon/cm^2/pulse. The work of Wittmershaus et al. [25] showed no change in the fluorescence decay of PSI-50 particles for excitation in the range 2×10^{12}–3×10^{15} photon/cm^2/pulse. We therefore assume annihilation effects are unimportant on the time scale of the present study.

The anisotropy data for PSI-40 particles up to a time delay of 40 ps (fig. 4) have a much lower raw initial anisotropy $R(0)$ (fig. 4c) value as a result of the finite resolution (1 channel = 100 fs) than do data detected with higher resolution (in fig. 3: 1 channel = 6.67 fs). Analysis of this data set shows that no additional anisotropy decay components in the 4–10 ps range are detectable with our current signal-to-noise ratio. This finding is in contrast to the single-color pump–probe measurements of Struve and coworkers [14]. The magic angle decay (fig. 4b) on the other hand does contain a short component of 5.4 ± 0.5 ps. As noted earlier, the anisotropy does not decay to zero but to a value of 0.1–0.2. This is in agreement with the observations of Struve and coworkers [14,15] and was ascribed by these workers to the intrinsic residual anisotropy of the PSI particles.

4. Discussion

Within the constraints imposed by our limited knowledge of molecular separations, strengths of intermolecular interactions, and of the various dephasing timescales in the PSI core antenna system, there are several alternative explanations for the ultrafast depolarization observed in this work. Kenkre and Knox [26] suggested that the inverse of the spectral linewidth provides an upper limit to the time for which coherent energy transfer would be possible. After this time, they suggest, Förster-type incoherent transport will be the appropriate model. Optical dephasing times for chromophores in solution are in the 10–60 fs range [27,28] and so an incoherent hopping picture would seem appropriate for our data in the case that the antenna molecules are weakly coupled with each other. On the other hand,

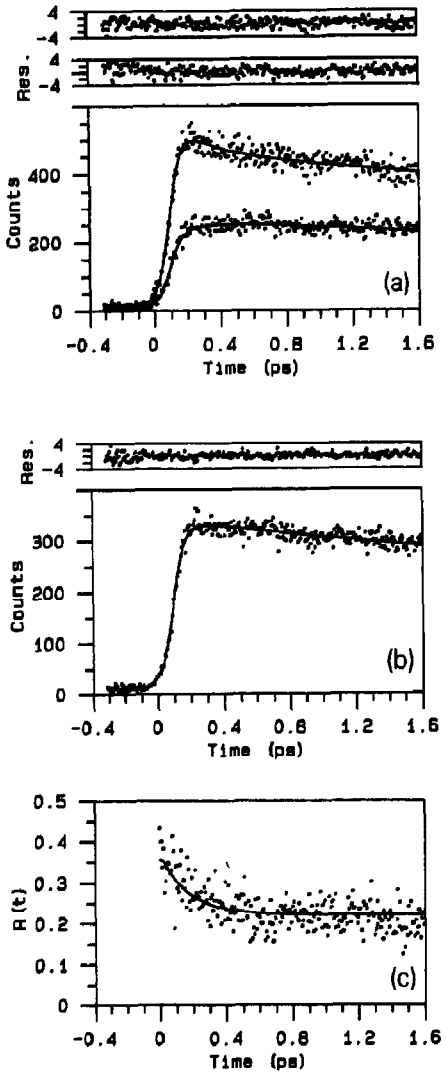

Fig. 3. Typical fluorescence data set and fit for PSI particles over a 2 ps scan with $\lambda_{ex}=650$ nm and $\lambda_{em}=730$ nm. The solid lines are fitted curves and the dots represent data. (a) Parallel (upper) and perpendicular (lower) data and fits. Residuals are shown above the data. Top panel is for parallel data and bottom panel is for perpendicular data. (b) Isotropic decay. (c) Raw anisotropy (dots) and the fit (solid line) with $r(t)=0.13\exp(-t/0.18\ \text{ps})+0.21$.

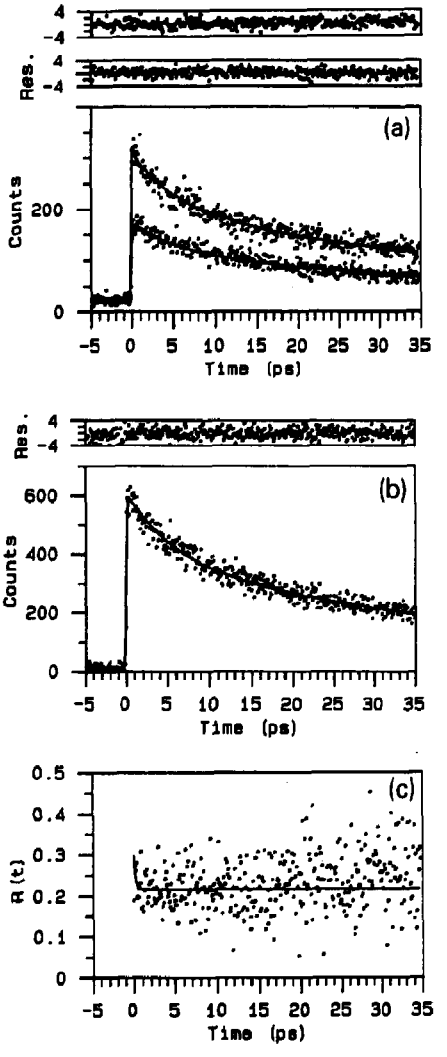

Fig. 4. Fluorescence data set and fit for PSI particles over a 40 ps scan with $\lambda_{ex}=650$ nm and $\lambda_{em}=730$ nm. The solid lines are fitted curves and the dots represent data. (a) Parallel (upper) and perpendicular (lower) data and fits. Residuals are shown above the data. Top panel is for parallel data and bottom panel is for perpendicular data. (b) Isotropic decay. (c) Raw anisotropy (dots) and the fit (solid line) with $r(t)=0.13\exp(-t/0.18\ \text{ps})+0.21$.

vibrational dephasing times in large molecules are much longer – typically in the 100 fs–5 ps range [29,30]. The influence of finite vibrational dephasing times on electron transfer reactions has been studied theoretically [31]. However, the effect of vibrational dephasing on energy transfer has not, to our knowledge, been investigated from a theoretical perspective. In the PSI core antenna, our results show that the ultrafast depolarization has no significant excitation and/or emission wavelength dependence, which is consistent with the small displacements of vibrational coordinates between the ground and excited states of Chl a observed spectroscopically [32]

provided that vibrational relaxation is fast. These small displacements make energy transfer much slower from higher vibronic level than lowest level and thus all excitation wavelengths correspond to essentially the same initial condition. Therefore we will take as the simplest model one involving Förster-type incoherent hopping between different Chl a molecules in the antenna. In this case the interpretation of the ultrafast fluorescence depolarization is straightforward: the polarization decays as a result of excitation hopping between differently oriented Chl a molecules [33–35]. If the distribution of nearest-neighbor orientations, averaged over the entire array, approximates a random distribution, the observed time constant (180 fs) corresponds roughly to the time of a single hop and is very similar to the estimate of Owens et al. [12]. If there is some correlation in the orientation of nearest neighbors, the observed decay time is an upper limit to the time for a single hop. The observed time is also very similar to the average single step transfer time required in simulations of spectrally disordered lattices chosen to mimic the PSI core antenna [5,12]. From the calculated Förster overlaps of Jia et al. [5], this timescale corresponds to an average separation of 11.5 Å between chromophores. Our result, however, stands in strong contrast with the conclusions from single-wavelength pump–probe studies that single step transfer times are in the range 3–7 ps for PSI-60 particles [14]. This discrepancy may not be real, however, as Struve and co-workers [15] have very recently reported an unresolvably fast decay in their two-color pump–probe experiments and concluded that their single-wavelength studies are not sensitive to the major energy transfer dynamics.

If a significant fraction of the antenna molecules are strongly coupled with each other, the interpretation of our short time data may be more complex. The description of the optical dynamics of strongly coupled vibronic systems is an active area of theoretical [31,36–38] and experimental [38] effort. In principle initial anisotropies ranging from 0.1 to 0.7 may be observed [37], in analogy to resonance Raman depolarization ratios [39]. The anisotropy would then be expected to contain "decay" terms reflecting the electronic dephasing timescale. In the present work our maximum initial anisotropy value is 0.34 ± 0.03. Although the effect is small, this appears to be systematically lower than for Chl a in solution and may represent the (unresolved) relaxation of delocalized states. However the low initial anisotropy may also arise from (anisotropic) reabsorption in the (local) optically dense antenna system. Further discussion of the role of delocalized excitons in this system must, however, await more detailed structural and spectroscopic information. We note, however, that if the 180 fs decay of anisotropy observed in these studies represents a dephasing process, and subsequent incoherent energy migration occurred on, say, a 1 ps timescale, we would expect to see depolarization on this timescale also. As noted earlier, within our current precision for fluorescence detected at 730 nm, we find no intermediate timescale anisotropy components.

Anisotropy decay components on longer timescales might also be expected if the core antenna were organized in a subunit model, with the slower components representing inter-subunit transfer. However, without a detailed structural model, the lack of a longer timescale decay does not allow us to exclude the possibility that, because of similar orientations, energy transfer between subunits may not cause significant depolarization. The subunit model does not seem consistent with what is known about the PSI core structure [9], or with our recent temperature- and wavelength-dependent picosecond studies of PSI [5,12,18]. Interestingly in systems which are known to possess subunit structures – LHCII [8] and the C-phycocyanin trimer [20], we do observe two timescales of fluorescence depolarization. In C-phycocyanin trimers using 605 nm excitation and 650 nm detection we find 800 fs and 20 ps components [20]. In LHCII with 650 nm excitation and 730 nm detection we find ≈ 200 fs and 4–7 ps anisotropy decay components [40]. The absence of a similar longer component in the PSI-40 particles, which do not have a structural subunit basis for Chl clusters, suggests that a subunit model [14] may be inappropriate for this system.

Finally, we turn to the topic of the timescale on which the initial excitation reaches an equilibrium or representative distribution among the inhomogeneous distribution of absorbers in the antenna. We have previously represented the PSI core antenna by using two types of inhomogeneity – a temperature independent continuous distribution of 200 cm^{-1}

taken from the hole-burning work of Small and coworkers [16] and a set of 5–7 spectral types. We can phenomenologically define a spectral evolution correlation function

$$C(t)=\frac{\nu(t)-\nu(\infty)}{\nu(0)-\nu(\infty)},$$

where $\nu(t)$ is the first moment of the fluorescence spectrum at time t. Simulations based on the same lattice used previously (Model 9 [5]) give $1/e$ times for $C(t)$ ranging from ≈ 1–4 ps depending on excitation wavelength. It thus seems very likely that the 5.4 ± 0.5 ps component observed in our magic angle decays at 730 nm corresponds to spectral equilibration. A thorough spectrally resolved study is needed to characterize this process fully.

5. Conclusion

We have experimentally observed the ultrafast energy transfer step in the PSI core antenna for the first time. An upper limit of 0.18 ± 0.04 ps for the average single transfer time was determined. This ultrafast process brings into focus many challenging questions: Does the energy transfer mechanisms involve strong, intermediate or weak coupling, or the combination of all three mechanisms? What are the roles of inhomogeneous broadening and of electronic and vibrational dephasing in the energy transfer process? What is the proper description of spatial and spectral organization for PSI core antenna system? Clearly both structural and time-resolved data are required to address these issues.

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