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Femtosecond Energy Transfer and Spectral Equilibration in Bacteriochlorophyll *a*—Protein Antenna Trimers from the Green Bacterium *Chlorobium tepidum*

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ABSTRACT Femtosecond energy transfer processes in a bacteriochlorophyll *a* - protein antenna complex from the green sulfur bacterium *Chlorobium tepidum* have been studied by one-color, two-color, and broadband absorption difference spectroscopy. Much of the spectral excitation equilibration in this antenna occurs with 350 to 450 fs kinetics. The anisotropy decay functions $r(t)$ exhibit two major lifetime components, 100 to 130 fs and 1.7 to 2.0 ps. The short component lifetimes may represent single-step energy transfer kinetics in this antenna; the long component is similar to the anisotropy decay observed in earlier picosecond pump-probe experiments.

INTRODUCTION

The trimeric bacteriochlorophyll *a*-containing protein found in green sulfur photosynthetic bacteria mediates electronic energy transfer between the peripheral antenna complex (known as the chlorosome) and the photochemical reaction center (Olson, 1980a). This protein (also known as the FMO protein after Fenna, Matthews, and Olson) has been extensively studied by spectroscopic, structural, and theoretical methods. It remains the only antenna complex with chlorophyll-like pigments whose 3-dimensional structure is known at high resolution.

The most extensively studied FMO protein has been the one isolated from *Prosthecochloris aestuarii*; its x-ray structure has been determined to 1.9 Å resolution (Matthews and Fenna, 1980; Tronrud et al. 1986). Each protein subunit encloses seven BChl *a* pigments; the subunits are tightly associated into trimers containing a total of 21 pigments. Spectral hole-burning studies (Johnson and Small, 1991) have established the presence of large exciton couplings (up to $\sim 200\ cm^{-1}$) among the 21 BChl *a* pigments in a trimer. Its absorption and CD spectra (Philipson and Sauer, 1972) have been realistically simulated in exciton calculations (Pearlstein, 1992; Lu and Pearlstein, 1993). The single-site transition wavelengths for the seven BChl *a* pigments in each subunit appear to vary considerably; Lu and Pearlstein (1993) estimated that their Q_y absorption maxima in the absence of resonance interactions would range from 780 to 820 nm.

Similar FMO proteins have been found in all green sulfur bacteria examined to date. The protein utilized in this study was isolated from the thermophilic green bacterium *Chlorobium tepidum* (Wahlund et al., 1991). Its room temperature absorption spectrum is essentially identical to those of other FMO proteins (Olson, 1980b; Blankenship et al., 1993). The gene for the protein from *C. tepidum* has been cloned and sequenced (Dracheva et al., 1992); its protein sequence is 78% identical to that of *P. aestuarii* (Daurat-Larroque et al., 1986). All of the pigment-coordinating residues in *P. aestuarii* are conserved in *C. tepidum*.

We are aware of few time-resolved experiments that have examined FMO trimer energy transfer kinetics time scales of less than ~10 ps. Lyle and Struve (1990) performed a one-color pump-probe anisotropy study of FMO trimers from *P. aestuarii* at 814 nm. Single-exponential fitting of the anisotropic decays yielded a 2.3-ps lifetime component, arising from energy transfer between BChl *a* pigments absorbing at this wavelength with nonparallel transition moments. No other lifetime components under 10 ps were discernible under the 2- to 3-ps laser autocorrelation function width. The time resolution has now been extended to ~100 fs, by using a pump-probe apparatus combined with a self-mode-locked Ti:sapphire laser (770–830 nm), and by using a femtosecond spectrometer employing 590 nm pump pulses and broadband continuum probe pulses. These experiments have yielded a wealth of detailed information on the femtosecond energy transfer kinetics in FMO trimers.

MATERIALS AND METHODS

FMO trimers were isolated from *C. tepidum* cells as described by Olson (1980b), with the following modifications. Fifty grams of wet-packed cells were suspended in 300 ml of 20 mM Tris/HCl buffer (pH 9.0) and sonicated 3 times for 3 min in a Branson Sonifier 350 cell disrupter (power setting 8); 3.5 M Na_2CO_3 was slowly added to 0.2 M final concentration, and the sonicate was stirred gently for 20 h in the dark at 4 C. Debris was removed by centrifuging at $10{,}000 \times g$ for 15 min; Na_2CO_3 was added to 0.4 M final concentration, and the solution was stirred for 20 h at 4 C. After centrifuging for 2 h in a 45-Ti rotor at 45,000 rpm, the resulting blue supernatant was carefully decanted, dialyzed against 20 mM Tris/HCl (pH 8.0), and applied to a 5.5 × 10 cm DEAE Sephacel column. The BChl *a* protein was eluted with 50 to 100 mM NaCl in the same buffer. Fractions were purity-assayed according to the spectral absorption ratio SAR = A_{267}/A_{371}. The pure BChl *a* protein exhibited SAR = 0.6, and ran as a single band on SDS-PAGE.

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The absorption spectrum showed maxima at 809, 602, 371, and 267 nm, with absorbance ratios 5.4:1:2.6:1.6, and is essentially identical to those of similar BChl *a* proteins isolated from other green sulfur bacteria (Blankenship et al., 1993).

The self-mode-locked Ti:sapphire laser was constructed after the design of Huang et al. (1992). In one-color experiments, tuning was provided by a single-plate intracavity birefringent filter, yielding ~120 fs fwhm laser pulse autocorrelations and 10 to 12 nm bandwidth at wavelengths between 770 and 830 nm. In two-color experiments (see below), the birefringent filter was omitted, yielding ~60-fs autocorrelations. The pump-probe apparatus was similar to one described by Causgrove et al. (1989), except that the lead molybdate acoustooptic modulators (AOMs) in the multiple modulation scheme (Anfinrud and Struve, 1986) were replaced with IntraAction AOM-80NR flint glass AOMs. In the one-color experiments, group velocity dispersion (GVD) in the pump-probe optics broadened the apparatus instrument function to ~260 fs fwhm. In the two-color experiments, this GVD was precompensated by two extracavity SF-10 glass Brewster prisms separated by 50 cm. Samples were housed at room temperature in a 7.6-cm diameter cell that rotated at ~1000 rpm. The femtosecond spectrometer (Taguchi et al., 1992) excited the sample in its Q_x band with ~200-fs pulses from a rhodamine 6G dye laser, and probed its transient absorption spectrum with white pulses generated in a continuum cell.

RESULTS

Fig. 1 shows one-color isotropic pump-probe profiles of FMO trimers, obtained using probe pulses polarized at the magic angle with respect to the pump polarization. The Ti:sapphire laser spectral width in the one-color experiments was ~10 nm. This bandwidth typically excites several ($\geq$3) exciton components in the Q_y band system (Lu and Pearlstein, 1993). At the two wavelengths shown (796 and 821 nm), the absorption difference signal is dominated at all times by a combination (PB/SE) of photobleaching (PB) and stimulated emission (SE). The 796-nm photobleaching decay exhibits a major 450-fs decay component, followed by a slower decay. The latter decay resembles the long-time kinetics observed by Blankenship et al. (1993) for FMO trimers in the absence of sodium dithionite. In contrast, the PB/SE decay observed at 821 nm (which excites the lowest-energy exciton bands in this antenna) exhibits *no* discernible femtosecond component, but displays kinetics similar to those of the slow components observed at 796 nm. Hence, the 450-fs PB/SE decay component at 796 nm corresponds to downhill transfer (spectral redistribution) of excitation from pigments excited at 796 nm to longer-wavelength pigments.

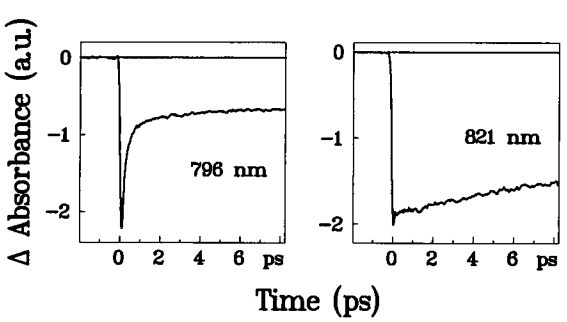

FIGURE 1 One-color isotropic absorption difference profiles for FMO trimers at 796 and 821 nm. Negative signals correspond to a combination of photobleaching and stimulated emission.

Two-color experiments were performed in the Q_y band system by removing the intracavity birefringent filter from the Ti:sapphire laser, generating 810 nm pulses with ~60 fs autocorrelation and ~30 nm spectral width. The pump and probe pulses were passed through bandpass interference filters (CVI Laser Corp.) centered at 800 and 820 nm, respectively. This produced light pulses with nonoverlapping spectral profiles ~7 nm wide; lifetime broadening correspondingly increased their cross-correlation width to ~210 fs fwhm. A two-color absorption difference signal excited at 800 nm and probed at 820 nm is shown in Fig. 2. Under present resolution and signal/noise, a 370 fs risetime feature is clearly present; the PB/SE decay over the next 8 ps can be fitted with a lifetime component in the tens of picoseconds. For comparison, a PB/SE signal simulated with prompt rise behavior and 34 ps decay time is also shown. Hence, we conclude that a major time scale for spectral equilibration of Q_y excitation in FMO trimers pumped at 796 to 800 nm is 350 to 450 fs.

A complementary view of this equilibration is given by the time evolution in the Q_y PB/SE spectrum following 590 nm excitation of FMO trimers in the Q_x band (Fig. 3). For delta-function excitation, these spectra would reflect a convolution of the $Q_x \rightarrow Q_y$ internal conversion (IC) kinetics with spectral equilibration of electronically hot Q_y excitations formed during IC. In part Fig. 3 *a*, the growth kinetics in the integrated PB/SE amplitude $|\Delta A|$ correspond approximately to the IC time scale. Little evolution is observed in this absorption difference spectrum between 0.6 and 8.0 ps (not shown), so that $Q_x \rightarrow Q_y$ IC appears to be essentially complete within 600 fs. The Q_y PB/SE spectrum clearly converges through downhill energy redistribution to an asymptotic spectrum within 400 to 500 fs. This is commensurate with the 350 to 450 fs spectral equilibration observed in the one- and two-color pump-probe experiments. The decay-associated spectra from biexponential

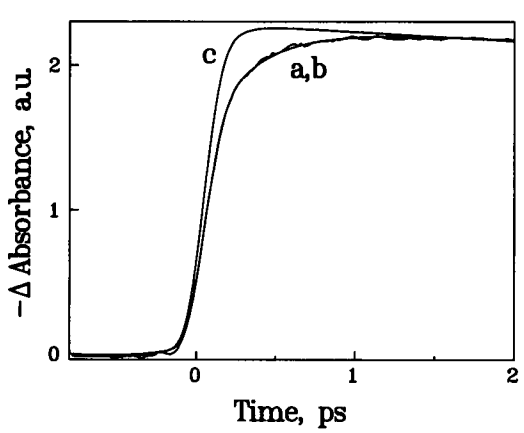

FIGURE 2 (*a*) Two-color isotropic absorption difference profile for FMO trimers excited at 800 nm and probed at 820 nm; (*b*) optimized biexponential fit to two-color profile, with 370 fs risetime and 34 ps decay time; (*c*) absorption difference profile simulated from convolution of 210 fs laser cross-correlation function with prompt molecular response. Curves (*a*) and (*b*) nearly coincide at most times, and correspond to noisy and smooth profiles, respectively.

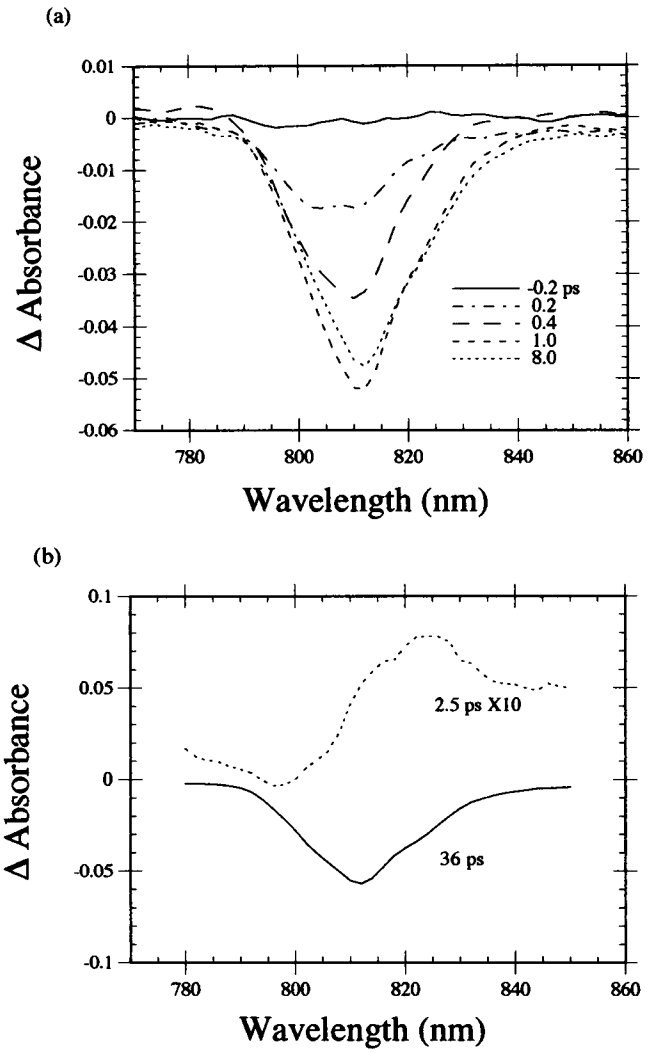

FIGURE 3 (*a*) Time-resolved near-infrared absorption difference spectra of FMO trimers excited at 590 nm. Delay times are (from top) −200 fs, 200 fs, 400 fs, 1 ps, and 8 ps; (*b*) Decay-associated spectra from biexponential global analysis of the difference spectra between 1 and 9 ps: 2.5 ps component (upper curve, multiplied by ×10 for clarity); 36 ps component (lower curve).

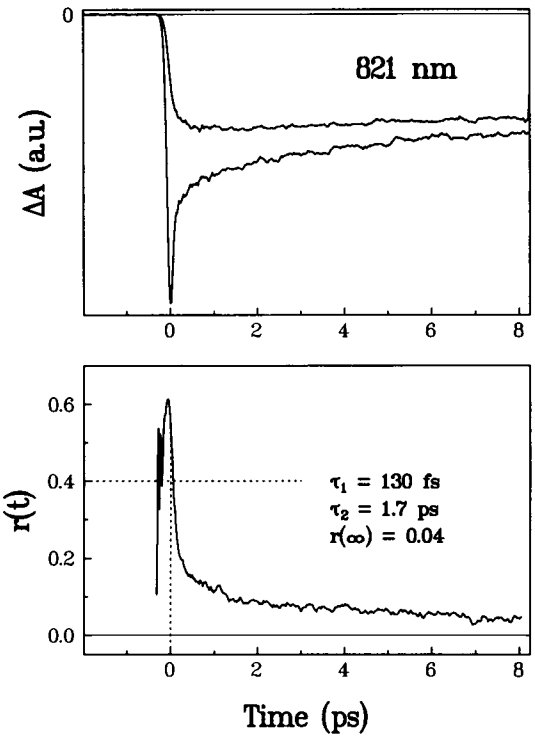

FIGURE 4 One-color anisotropic absorption difference profiles $\Delta A_{\parallel}(t)$ and $\Delta A_{\perp}(t)$ at 821 nm; (*b*) anisotropy decay function $r(t)$. Lifetimes are from optimized biexponential fit to the anisotropy decay (see text).

global analysis of these profiles from 1 to 9 ps are shown in Fig. 3 *b*. These reveal a small-amplitude, bipolar 2.5 ps DAS component, associated with downhill equilibration between spectral forms at ~800 and ~820 nm. Hence, the spectral equilibration occurs via picosecond as well as femtosecond processes.

One-color anisotropic pump-probe profiles are shown for 821 nm in Fig. 4, along with the computed anisotropy function $r(t) = (\Delta A_{\parallel} - \Delta A_{\perp})/(\Delta A_{\parallel} + 2\Delta A_{\perp})$. A biexponential fit to $r(t)$ yields lifetime components 130 fs and 1.7 ps. Similar anisotropy decay components are found at other wavelengths (e.g., 100 fs and 2.0 ps at 796 nm). In each case, the long anisotropy decay component resembles the 2.3 ps anisotropy decay found at 814 nm in earlier work (Lyle and Struve, 1990). They also resemble the 2.5-ps DAS component associated with slow downhill energy transfer. The 100 to 130 fs components may correspond to single-step energy transfer in FMO trimers. Relaxation between exciton components having contrasting transition moment directions would also cause anisotropy decay.

The value of the initial anisotropy $r(0)$ is of interest, because it can theoretically exceed 0.4 if several exciton components are coherently excited (Knox and Gülen, 1993). In the presence of couplings between BChl *a* chromophores belonging to different subunits, each of the seven Q_y exciton levels in one antenna protein subunit becomes split into three sublevels in the trimer (Johnson and Small, 1991). Of these, a nondegenerate level (with Einstein absorption coefficient $B_{\parallel}$) is polarized along the trimer symmetry axis, and a doubly degenerate pair (with Einstein coefficient $B_{\perp}$) are polarized in the trimer plane. If all three components are uniformly excited with a broadband laser, a PB/SE anisotropy calculation analogous to one in a theoretical fluorescence depolarization study by Rahman et al. (1979) predicts $r(0) = (7 + 2x^2 + 6x)/(10 + 5x^2)$, where $x = B_{\parallel}/B_{\perp}$ is the ratio of out-of-plane to in-plane absorption coefficients (Struve, 1993). Hence, $r(0)$ is generally predicted to lie between 0.4 and 1.0, approaching the lower limit only when a single, linearly polarized exciton transition strongly dominates ($B_{\parallel} >> B_{\perp}$). While the pump-probe signals at $t = 0$ are obscured by the coherent coupling artifact, it is still possible to draw inferences about $r(0)$ from the present anisotropy decay. Since the apparatus instrument function (broadened to ~260 fs by GVD in this one-color experiment) is considerably narrower than the coherent spike (~120 fs fwhm), the

pump-probe signals at negative times from ~−200 to −100 fs are dominated by FMO absorption difference signals rather than by coherent coupling artifacts. At such times, the statistically significant anisotropy function appears to be greater than 0.5 at 821 nm (Fig. 4). In this context, it is interesting that the experimental anisotropy function decays to <0.4 within ≤100 fs at all of the wavelengths studied to date (not shown). Hence, the initial coherence in the laser-prepared superimposition of exciton states appears to become lost within this time scale.

Some of the early-time energy transfer events in FMO trimers thus appear to have been revealed with unprecedented detail and clarity. It is tempting to speculate that the femtosecond and picosecond lifetime components may originate from energy transfers within and between monomer subunits, respectively. Since this complex is structurally and spectroscopically the best characterized of all photosynthetic antennae, mechanistic assignments of these lifetime components may soon become possible, pending numerical simulations of its energy transfer kinetics.

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