Isolated-Chromophore and Exciton-State Photophysics in *C*-Phycocyanin Trimers

Ruth E. Riter,† Maurice D. Edington, and Warren F. Beck*

Department of Chemistry, Vanderbilt University, 5134 Stevenson Center, P.O. Box 1822-B, Nashville, Tennessee 37235

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We have employed femtosecond pump−probe spectroscopy to examine the photophysics of *C*-phycocyanin, a photosynthetic light-harvesting protein located in the rods of the phycobilisome in cyanobacteria. The experiments were intended to focus on the photophysics of the paired α84 and *β*84 phycocyanobilin chromophores that are present in *C*-phycocyanin's C_3-symmetric trimeric aggregation state. The time-resolved pump−probe absorption-difference spectrum exhibits initially narrow photobleaching/stimulated-emission holes that broaden on the 200 fs time scale but shift 250 cm^{-1} to lower energy on the ∼50 fs time scale. The line broadening arises primarily from intramolecular vibrational redistribution, as observed previously in preparations of the α subunit (*J. Phys. Chem.* **1996**, *100*, 14198−14205), which contains only one phycocyanobilin chromophore. The pump−probe anisotropy in *C*-phycocyanin trimers exhibits decay components on the 20−60 and ∼700 fs time scales when the probe beam is centered on the 640 nm region, where a rising stimulated-emission signal is observed owing to the red shift of the time-resolved pump−probe spectrum. The initial anisotropy is significantly larger than 0.4, which strongly suggests the presence of an initially delocalized state involving the α84 and *β*84 chromophores. The fast red shift of the pump−probe spectrum, then, can be assigned to interexciton-state relaxation between the two exciton states of the coupled α84−*β*84 pairs.

Introduction

There has been a great deal of recent interest in the role of delocalized excited states[1−3] in photosynthetic light-harvesting proteins. Our work in this area has been motivated by the possibility that dimers or larger clustered arrays of chromophores optimize the delivery of excitation energy to the photosynthetic reaction center. The cyanobacterial phycobiliproteins[4,5] *C*-phycocyanin and allophycocyanin are especially attractive systems in which the role of delocalized excited states in chromophore dimer systems can be explored. In both proteins, the tertiary and quaternary structure organizes the binding sites for phycocyanobilin (open-chain tetrapyrrole) chromophores so that dimer systems are formed across polypeptide interfaces.[6−10] This feature allows us to examine the intrinsic photophysics of the antenna chromophore and its surrounding protein matrix[11] in parallel with studies on the photophysics pertaining to dimer coherence[12−16] or short-range Förster energy transfer,[17,18] whichever is relevant, by manipulating the aggregation state.

C-Phycocyanin and allophycocyanin form almost identical C_3-symmetric, ringlike homotrimers of α,*β* polypeptide monomers in solution. (See the MOLSCRIPT[19] representation of the *C*-phycocyanin structure shown in Figure 1.) The trimeric aggregation state places the α84 and *β*84 chromophores within 1.2 nm (the shortest pyrrole−pyrrole separation) of each other.[6−10] In allophycocyanin, the maximum of the continuous ground-state absorption spectrum exhibited by the chromophore complement is shifted to lower energy by ∼750 cm^{-1} upon aggregation of monomers into trimers. In addition, the spectrum exhibits a partially resolved splitting. The red shift and splitting have been attributed to the formation of α84−*β*84 chromophore−dimer (exciton) states;[20−23] however, changes in the chromophore environments upon aggregation of monomers into trimers also contribute to the spectral changes.[10,23] Although the continuous ground-state absorption spectrum exhibited by *C*-phycocyanin in the trimeric aggregation state does not unambiguously indicate any structure, the spectrum is made complex by the presence of the extra chromophore in the *β* subunit, *β*155, which is held well separated from the other chromophores. Note that the structure observed in the absorption spectrum of allophycocyanin arises from the exciton splitting and from the α84−*β*84 site-energy gap,[23] so a smaller site-energy gap for the α84 and *β*84 chromophores in *C*-phycocyanin might account for the lack of resolved structure.

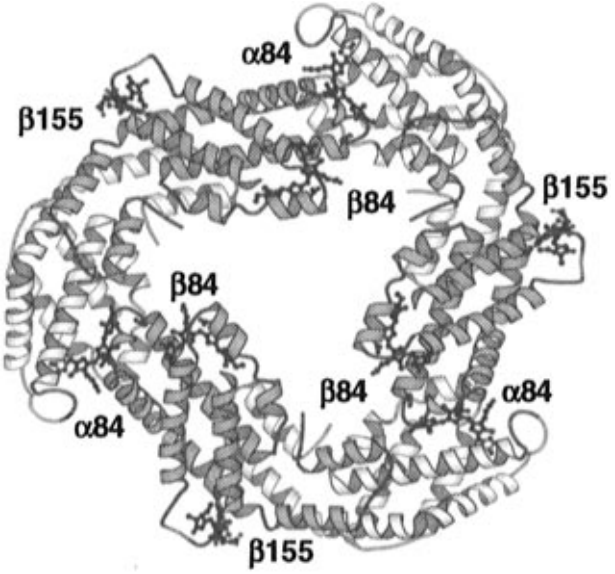

Figure 1. MOLSCRIPT[19] ribbon representation of the structure of *C*-phycocyanin trimers from *Fremyella diplosiphon*, as rendered from the Brookhaven PDB file *1cpc* determined by Huber and co-workers.[9] The phycocyanobilin chromophores are displayed in a ball-and-stick format; the labels refer to the cysteine-derived thioether ligation points for the chromophores in the α and *β* subunits.

The photophysics exhibited by the α84−*β*84 chromophore dimers in allophycocyanin can easily be distinguished from that

† Present address: Department of Chemistry, Colorado State University, Fort Collins, CO 80523.

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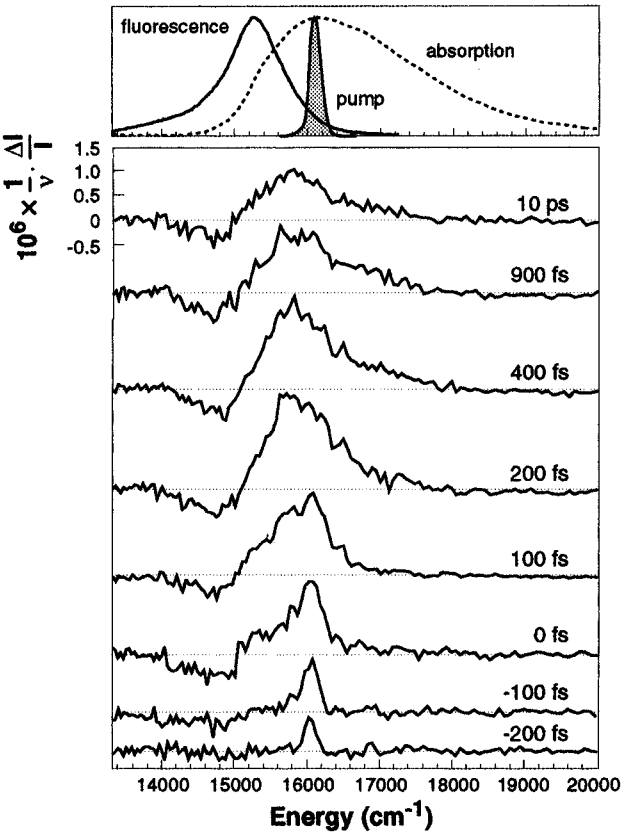

Figure 2. Continuous ground-state absorption and fluorescence-emission spectra (top panel) and time-resolved pump−probe absorption-difference spectra at various probe delays (bottom panel) obtained at room temperature (20 °C) with *C*-phycocyanin trimer preparations. The intensity spectrum exhibited by the pump pulses is shown superimposed in the top panel on the absorption and fluorescence-emission spectra. The absorption spectrum was recorded with a 2 nm spectral band-pass; the uncorrected fluorescence-emission spectrum was obtained with excitation at 560 nm (17 860 cm^{-1}) and was recorded with 4 nm spectral band-pass. The time-resolved pump−probe spectra were recorded with 4 nm spectral band-pass.

exhibited by a single phycocyanobilin chromophore, as in isolated α subunits of *C*-phycocyanin. The time-resolved pump−probe absorption-difference spectrum obtained with the α subunit exhibits initially narrow photobleaching/stimulated-emission (PB/SE) features centered near the pump spectrum; the PB and SE components exhibit line-broadening dynamics on the <200 fs time scale, and a dynamic Stokes shift of the SE component occurs on the <100 fs time scale. The time evolution of the spectrum is assigned to intramolecular vibrational redistribution (IVR) and to protein-matrix solvation dynamics.[11,24] In allophycocyanin trimers, the absorption-difference spectrum[23] exhibits a PB/SE band that is well red-shifted from the pump spectrum's position, even at the earliest probe delays.[23] This finding indicates that the photophysics in allophycocyanin trimers are dominated by interexciton-state relaxation, a radiationless transfer of population between exciton states.[12]

Since the *C*-phycocyanin and allophycocyanin structures are very similar, the α84−β84 chromophore pairs in *C*-phycocyanin trimers would be expected to exhibit comparable photophysics. The fluorescence-emission spectrum of *C*-phycocyanin deviates markedly from mirror symmetry with respect to the absorption spectrum in terms of position and width (Figure 2, top panel), as is also observed in allophycocyanin, so it is natural to suggest the presence of a radiationless decay mechanism or a Förster energy-transfer process that quenches the fluorescence of higher-energy chromophore states. The latter interpretation is supported by the results of one-color femtosecond pump−probe experiments by Gillbro and co-workers[25] and by the results of femtosecond fluorescence up-conversion experiments by Xie et al.[26] Calculations by Sauer and Scheer[27−29] anticipate that Förster energy transfer should occur between the α84 and β84 chromophores in *C*-phycocyanin trimers on the ∼0.5 ps time scale.

In this contribution, we discuss the subpicosecond time evolution of the time-resolved pump−probe absorption-difference spectrum exhibited by *C*-phycocyanin trimers at room temperature. A fast red shift of the central PB/SE band occurs on the ∼50 fs time scale, but no spectral evolution consistent with a Förster energy-transfer process on the subpicosecond time scale is observed. Two-color pump−probe anisotropy decays report the presence of an initially delocalized state involving α84−β84 dimers in terms of a biphasic decay from a large initial anisotropy. As we observed in allophycocyanin,[22] the fast phase of the anisotropy decay occurs on a similar time scale as the red shift of the time-resolved pump−probe spectrum. These findings show that interexciton-state relaxation occurs between the exciton states of the α84−β84 chromophore dimers in *C*-phycocyanin.

Experimental Section

Sample Preparation. *C*-Phycocyanin trimers were isolated from cultures of the AN112 mutant of the cyanobacterium *Synechococcus* PCC 6301 using previously described methods.[4] Allophycocyanin and *C*-phycocyanin were purified using low-pressure ion-exchange chromatography on DEAE-cellulose[4] and DEAE-Bio-Gel A (Bio-Rad) columns. Isolated *C*-phycocyanin trimers were stored at 4 °C as concentrated solutions (>2 absorbance, as measured at 620 nm in a 1 mm cuvette) in a 100 mM phosphate buffer solution at pH 5.5 in the dark. On the day of an experiment, *C*-phycocyanin preparations were dialyzed against a 100 mM phosphate buffer solution at pH 7 and were then concentrated over a PM-30 membrane in an Amicon ultrafiltration cell at 4 °C. The final sample absorption was adjusted to 0.4−0.5 at 620 nm, as measured in a quartz cell with a 1 mm path length.

C-Phycocyanin preparations at pH 7 were analyzed at room temperature on an HPLC size-exclusion column (Rainin Hydropore-5-SEC) at injection concentrations comparable to those used in femtosecond pump−probe experiments. The chromatograms were compared to those obtained under the same conditions with gel-filtration protein standards (Bio-Rad). The largest peak in the chromatogram obtained with *C*-phycocyanin preparations (not shown) corresponds to the trimeric $(\alpha\beta)_3$ aggregation state, with a smaller peak corresponding to the hexameric $(\alpha\beta)_6$ state. We estimate that our preparations are composed of a ∼85/15 ratio of trimers/hexamers under the conditions used. In this paper, we refer to the C-phycocyanin preparations used in femtosecond pump−probe experiments as trimers, but it should be understood that the preparations are equilibrium mixtures of trimers and hexamers, in the proportions mentioned above.

Ground-state absorption spectra were obtained with a Hitachi U2000 spectrophotometer, which was controlled by LabVIEW (National Instruments) routines. Fluorescence-emission spectra were obtained with a SLM Aminco-Bowman Series 2 spectrometer in the laboratory of Professor W. M. LeStourgeon, Department of Molecular Biology, Vanderbilt University.

Femtosecond Spectroscopy. The femtosecond laser spectrometer used in time-resolved pump−probe experiments consists of an amplified colliding-pulse, mode-locked (CPM) dye laser and a modified Michelson pump−probe interferometer,

as described in detail previously.[11,22] Fifteen percent of the output of the amplified CPM laser (~2 μJ/pulse at 3 kHz) was injected into the pump arm of the pump−probe interferometer. The pump pulses exhibited 80−85 fs widths (assuming sech^2 pulse shapes) at the sample position. The spectrum of the pump pulses was centered at 620 nm and was ~6-nm wide, as measured with a grating spectrometer with a 4 nm band-pass. A femtosecond continuum, generated in flowing ethylene glycol held in a cell of 3 mm path length (Clark Instrumentation) using 85% of the output of the amplified CPM laser, was injected into the probe arm of the interferometer. Wavelength-resolved pump−probe measurements over a 450−800 nm (22 200−12 500 cm^{-1}) region could be conducted with the continuum probe light. The pump and probe beams were focused by the same 5 cm achromat onto the sample position. Pump−probe cross-correlation measurements performed with a KDP crystal (of 300 μm thickness) at the sample position yielded widths of about 150 fs at most probe wavelengths. Dichroism-free pump−probe signals were obtained with a 45° pump−probe polarization geometry with an analyzer polarizer in the transmitted probe beam set at the "mystic" angle (63.44°)[30] with respect to the pump beam.

The group-delay dispersion of the probe beam was corrected by programming a computer-controlled translation stage (Nanomover, Melles Griot) in the pump beam's delay arm to null the arrival delay for a given probe wavelength. The wavelength dependence of the group delay was determined by frequency-resolved pump−probe cross-correlation measurements[31] and by nonresonant optical Kerr effect experiments[32] conducted with the sample cell filled with sample buffer solution. The program for the pump-beam's translation stage employed a third-degree polynomial function to allow for compensation of the small degree of nonlinearity that was detected in the wavelength dependence of the group delay.

Anisotropy experiments were conducted using a discrete two-color technique, exactly as described previously.[22] In short, a three-cavity Fabry-Perot interference filter (Omega optics) was placed in the probe beam of the spectrometer described above, and the group-delay dispersion in the probe beam was compensated after the filter with a pair of SF-10 prisms.The probe beam's polarization was oriented 45° with respect to that of the pump beam; polarized transients were obtained by orienting the post-sample analyzer polarizer in the probe beam to select the desired polarization component of the transmitted probe beam with respect to the plane of polarization of the incident pump beam. The grating spectrometer and detection system used in to obtain time-resolved pump−probe spectra was employed in the anisotropy experiments to detect the analyzed probe beam. The proper performance of this apparatus in control experiments with dilute laser dye solutions (nile blue) was verified prior to work with preparations of *C*-phycocyanin trimers using the same optical conditions, as has been previously discussed.[22]

Samples were held in a fused-silica flow cuvette (1 mm path length). A total of 1−1.5 mL of sample solution was recirculated through the sample cuvette, with the sample reservoir held at ambient temperature (20 °C). A comparison of ground-state absorption spectra before and after the pump−probe experiments showed that permanent photobleaching of the phycobiliprotein preparations did not occur as a result of exposure to the laser light.

Time-resolved spectra were obtained with pump and probe energies of 50 and 25 nJ/pulse, respectively; neutral density filters were used to adjust the pump and probe beam intensity at the sample position. The anisotropy experiments were conducted with the pump and probe beams attenuated to 7 and 0.7 nJ/pulse, respectively. The pump-energy dependence of isotropic transients was characterized over a wider pump energy range, from 0.26 to 70 nJ/pulse.

Results

Figure 2 (bottom panel) shows a series of time-resolved pump−probe spectra obtained at room temperature with preparations of *C*-phycocyanin trimers at various probe delays. The top panel of Figure 2 shows the continuous absorption and fluorescence-emission spectra for the trimer preparations superimposed with the spectrum of the pump pulses. The initial spectrum, observed as the pump pulse just begins to enter the sample (−200 fs), exhibits a hole spectrum of net PB/SE character centered at the position of the pump-pulse spectrum. Over the next 400 fs of delay, the PB/SE hole broadens considerably to both the red and the blue and shifts to the red by ~250 cm^{-1}. At the same time, the PB/SE band shape evolves from a roughly symmetrical shape centered around the pump-pulse spectrum to an asymmetrical band with a maximum in the 15 600−15 800 cm^{-1} region and a broad tail to higher energy. The rise of intensity in the 15 600 cm^{-1} region is complete at a delay of 200 fs, whereas the line broadening continues for a longer period of time. Note that the 400 fs spectrum crosses the base line on the blue side of the central PB/SE band at ~18 250 cm^{-1}, whereas the 200 fs spectrum crosses the base line at only ~17 800 cm^{-1}; thus, the photobleaching portion of the spectrum has broadened considerably over the additional 200 fs of delay.

Note that the spectra shown in Figure 2 exhibit a broad region of net excited-state absorption (ESA) near 15 000 cm^{-1} and to lower energy. The spectra observed with isolated α subunits of *C*-phycocyanin[11] and with allophycocyanin trimers[23] exhibit similar regions of net ESA flanking the PB/SE peak on both the red and blue sides. The spectra shown in Figure 2 are likely, then, to incorporate contributions from a single, very broad, $S_1 \rightarrow S_n$ ESA band that spans the entire PB/SE region.

Over the 500 fs−10 ps time scale, the entire pump−probe spectrum obtained with *C*-phycocyanin trimers decays somewhat in intensity without changing very much in shape. The shape of the spectrum obtained at a delay of 900 fs is quite similar to that observed at a delay of 10 ps, as shown in Figure 2, except for the decrease in intensity across the spectrum, a small broadening, and a very modest red shift (<100 cm^{-1}) of the peak maximum. Discrete two-color pump−probe transients recorded with pump energies ranging from 0.26 to 70 nJ/pulse show that the magnitude of this overall decrease in signal intensity is weakly pump-energy-dependent. Some of the decrease in the peak intensity over the 500−1000 fs delay region can be assigned to the line broadening, and a portion of the decay can be attributed to fluorescence emission on the nanosecond time scale.[33] The pump-energy-dependent portion of the decay probably involves singlet−singlet annihilation processes,[2,34] but we have not performed experiments over a broad enough range of energies to allow a detailed modeling.

Figure 3 describes the time evolution of the center frequency, ν_0, and line width at half-maximum, $\Delta\nu$, of the central net PB/SE portion of the time-resolved pump−probe spectrum exhibited by *C*-phycocyanin trimers. At the earliest observed probe delay, ν_0 is near the pump energy (16 130 cm^{-1}); a shift to the red over a ~250 cm^{-1} range occurs well within the full width of the instrument response function (Figure 3, top panel). The line width $\Delta\nu$ expands with a roughly exponential time course, reaching a final value of ~900 cm^{-1} with a time constant τ of 241 fs (Figure 3, bottom panel). For purposes of comparison,

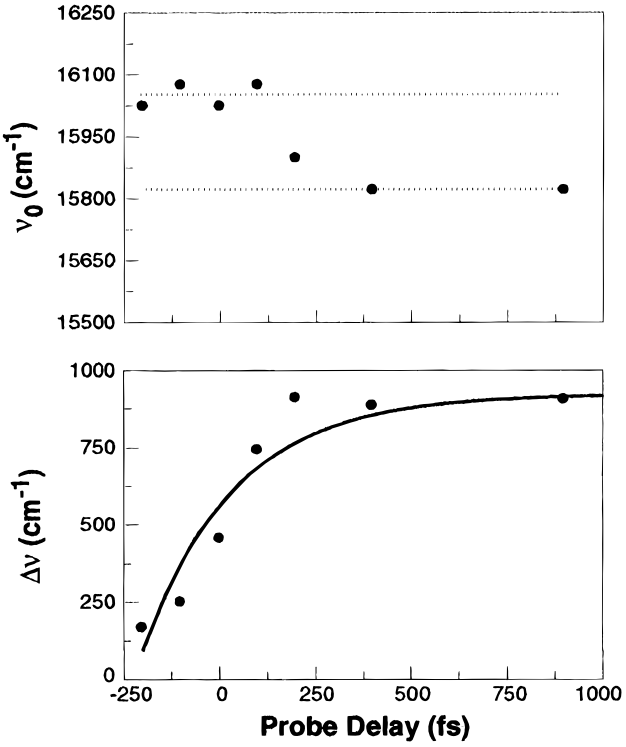

Figure 3. Time evolution of the center frequency, ν_0 (top panel) and line width, $\Delta\nu$ (bottom panel), of the time-resolved pump−probe absorption-difference spectrum exhibited by *C*-phycocyanin trimers (Figure 2). In the top panel, the horizontal dashed lines are intended to suggest the approximate initial and final positions of the PB/SE maximum. In the bottom panel, the superimposed curve represent a fit to the model $c_0e^{-t/\tau} + c_1$ obtained from a nonlinear least-squares regression analysis with $c_0 = 357 \pm 87$ cm^{-1}, $\tau = 241 \pm 63$ fs, and $c_1 = 927 \pm 57$ cm^{-1}.

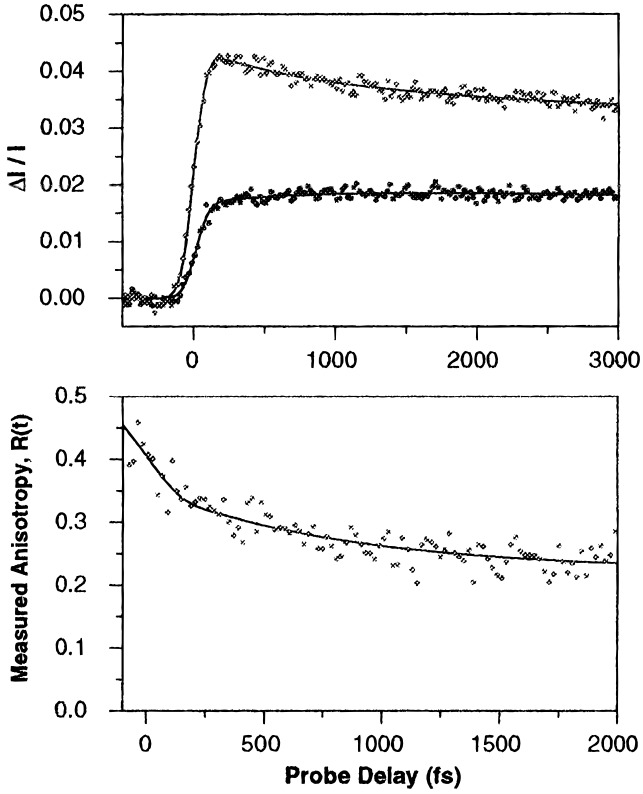

Figure 4. Polarized two-color pump−probe transients and *measured* anisotropy *R(t)* observed at room temperature (20 °C) with *C*-phycocyanin trimer preparations, obtained with pump pulses at 620 nm (16 130 cm^{-1}) and probe pulses at 640 nm (15 625 cm^{-1}). The two pump−probe transients (top panel) were obtained with the analyzer polarizer in the transmitted probe beam oriented parallel (top trace) and perpendicular (bottom trace) with respect to the polarization of the pump beam. *R*(*t*) (bottom panel) was computed directly from the data points for the parallel and perpendicular traces. Superimposed on the data points in both panels are fitted curves obtained using a global iterative reconvolution program, as discussed in the text. The isotropic function *K*(*t*) was $\sum_i a_i \exp(-t/\tau_i)$, where $a_1 = (1.1 \pm 0.05) \times 10^{-2}$, $\tau_1 = 12600 \pm 100$ fs, $a_2 = (-3.2 \pm 1.5) \times 10^{-3}$ (a rising component), $\tau_2 = 35 \pm 20$ fs, $\tau_3 = 1.5$ ns (fixed), and $a_3 = (1.5 \pm 0.05) \times 10^{-2}$. The *fitted* anisotropy function $r(t)$ was $\sum_i \beta_i \exp(-t/\phi_i)$, with $\beta_1 = 0.12 \pm 0.02$, $\phi_i = 690 \pm 130$ fs, $\beta_2 = 0.134^{-0.05}_{+0.13}$, $\phi_i = 35 \pm 20$ fs, $\beta_3 = 0.22 \pm 0.02$, and $\phi_i = \infty$ (a nondecaying offset). Uncertainties were estimated by examining the variation of the global χ^2 function as a given parameter was varied, with the other parameters fixed.

the PB and SE contributions to the spectrum exhibited by isolated α subunits were observed to broaden with time constants of 234 and 119 fs, respectively.[11]

The top panel of Figure 4 shows a set of polarized pump−probe transients obtained with *C*-phycocyanin trimers using a discrete two-color technique.[22] The focus of the experiment was on the rise of the net PB/SE signal in the 15 600−15 800 cm^{-1} region, where the PB/SE maximum eventually arises. The pump intensity used in experiments of this type was chosen to be low enough that the pump-energy-dependent portion of the decay observed in isotropic two-color transients was minimized but still high enough to obtain an adequate signal/noise ratio in the polarized transients. Superimposed on the transients shown in Figure 4 are fitted curves obtained with an iterative reconvolution procedure. As discussed previously in our study of the anisotropy decays observed in allophycocyanin,[22] polarized pump−probe transients can be analyzed using a method advanced by Cross and Fleming[35] that involves simultaneously fitting the perpendicular and parallel transients using a single model incorporating isotropic and anisotropic functions, *K*(*t*) and *r*(*t*), respectively (see the legend to Figure 4). The analysis compensates for the instrument response function using an iterative reconvolution approach; Bevington's[36] grid-search algorithm for nonlinear optimization and a convolution routine written in our laboratory were employed. The isotropic function *K*(*t*) exhibits a component on the ∼20−60 fs time scale accounting for about one-half of the intensity of the rise; the decay involves a weak 12 ps component that terminates in a long-lived (on the time window of the experiment) offset. This offset is assigned to the 1.5 ns S_1-state lifetime of the phycocyanobilin chromophore in *C*-phycocyanin.[33]

The lower panel of Figure 4 shows the *measured* pump−probe anisotropy $R(t) = (A_{\parallel}(t) - A_{\perp}(t)/(A_{\parallel}(t) + 2A_{\perp}(t))$, as calculated directly from the parallel and perpendicular transients, $A_{\parallel}(t)$ and $A_{\perp}(t)$, respectively, superimposed with the fit function obtained from the iterative reconvolution analysis. *R*(*t*) clearly exhibits two regions of anisotropy decay; note the break in the decay in the delay region near 250 fs from the initial fast component to a slower component that persists out to nearly 2 ps of delay. Accordingly, the best model for the anisotropy *r*(*t*) contains two exponentials that decay to a long-lived offset $r(\infty) = 0.22$. Owing to covariance, the magnitude of the initial anisotropy is coupled to the time constant of the fast component. Acceptable fits were obtained with time constants ranging from 20 to 60 fs for the fast component; the slow component exhibits a time constant of ∼700 fs. The latter time constant is quite similar to that obtained previously by Gillbro and co-workers[25] from one-color pump−probe experiments and by Xie et al.[26] from fluorescence up-conversion experiments.

Discussion

The time evolution of the time-resolved pump−probe spectrum exhibited by *C*-phycocyanin trimers should be understood in terms of overlapping[33,37−39] spectral responses involving the photophysics of isolated and coupled chromophores: the β155 chromophores and the α84−β84 chromophore−dimer systems, respectively. On the basis of the results of comparable experiments on α subunits,[11] we have good reason to believe that the phycocyanobilin chromophores in *C*-phycocyanin undergo IVR and protein-matrix solvation dynamics on the <200 fs time scale. Both of these phenomena contribute to the broadening of the narrow PB/SE holes that are initially observed in *C*-phycocyanin trimers, but vibrational relaxation and solvation dynamics should additionally cause a red shift of the stimulated-emission component of the spectrum. Additional photophysics, owing to energy transfer between uncoupled chromophores or radiationless decay between the exciton levels of coupled chromophores, would be superimposed on the single-chromophore dynamics. Energy transfer between the β155 chromophores and the other chromophores in a trimer occurs on the >40 ps time scale because β155 is separated from its nearest neighbor by >3 nm.[37,38] Accordingly, only the α84 and β84 chromophores can be implicated in any subpicosecond energy-transfer processes that occur in *C*-phycocyanin trimers.

The series of pump−probe spectra shown in Figure 2 do not exhibit a large spectral evolution on the >500 fs time scale that evidences Förster energy transfer between the α84 and β84 chromophores. Aside from line broadening, the subpicosecond isotropic spectral dynamics of the largest amplitude have to do with a ∼50 fs formation of a 250 cm^{-1} red-shifted PB/SE maximum in the 15 600−15 800 cm^{-1} region. The two-color pump−probe experiment described in Figure 4 shows that a rising component with a time constant in the 20−60 fs range accounts for about one-half of the total isotropic intensity in that region of the spectrum. Interexciton-state relaxation of population between the exciton states of the α84−β84 chromophore dimers would account for a red shift on the observed time scale, as discussed in the Introduction. The only other explanation for spectral evolution on this time scale is a dynamic Stokes shift of the SE contribution to the spectrum owing to transient solvation, arising from any of the three types of chromophores. Unfortunately, the pump−probe spectra alone do not allow us to distinguish between these two possibilities because of the overlapping contribution of the spectrum from the β155 chromophore.

The anisotropy information obtained from the two-color pump−probe experiment, however, indicates that a delocalized state is initially prepared by the 620 nm pump pulses. The initial anisotropy is significantly larger than the value of 0.4 associated with photoselection of an isolated transition dipole. The theory described by Wynne and Hochstrasser[13,15] and Knox and Gülen[12] describes how the fluorescence anisotropy to be expected from a coherently excited pair of chromophores should exceed 0.4, owing to interference phenomena associated with emission originating from the two exciton states. In the current pump−probe experiments, comparable effects would arise from stimulated emission from the exciton states. Matro and Cina[16] show that the anticipated initial anisotropy is described by $r(0) = 0.4 + 0.3(1-\cos^2\phi)/(1+\cos^2\phi)$, where ϕ is the angle between the transition dipole moments for the two chromophores in a dimer. Given the 65° angle estimated between the transition dipole moments for the α84 and β84 chromophores in a given dimer (see Figure 1),[6−8] the expected initial anisotropy $r(0)$ would be close to 0.6. Only about one-half of the prepared population, however, will be centered on the α84 and β84 chromophores because of the overlap of the ground-state absorption spectra.[38] The photoselected population of the β155 chromophore that also contributes to the pump−probe signal would contribute an initial anisotropy of 0.4, so the initial anisotropy expected from *C*-phycocyanin trimers would be in the range of ∼0.5. The estimated initial anisotropy in *C*-phycocyanin trimers obtained from the iterative reconvolution analysis is $0.47^{-0.09}_{+0.17}$, which places the center of the confidence interval in the appropriate range expected by theory.

As suggested by the theory and calculations reported by Matro and Cina[16] and from the analysis of the Wynne and Hochstrasser/Knox and Gülen theory by van Amerongen and Struve,[40] the initial fast decay of the anisotropy is associated with population dynamics between the exciton levels. The slower portion (700 fs time constant) of the anisotropy decay involves equilibration of population between the site states at a rate that would be comparable to that expected from Förster theory. The calculated anisotropy decays reported by Matro and Cina employed parameters appropriate for a simplified description of the α84−β84 chromophore dimers in *C*-phycocyanin trimers. The simulations are quite consistent with our experimental results, except that, as with allophycocyanin trimers,[22] the oscillations in the anisotropy that should arise from coherent energy transfer between the two chromophores are not observed. Matro and Cina's work shows that rapid damping of the population dynamics can arise in the presence of a thermal bath that mediates dephasing. Our results, then, are consistent with the previous finding that there is an inertial phase of protein-matrix solvation dynamics in the phycobiliproteins that operates on the <100 fs time scale.[11,24]

The results of this paper support the suggestion that the α84−β84 chromophore dimers in allophycocyanin and *C*-phycocyanin exhibit analogous photophysics. The main differences in the two systems arise, as proposed previously by Huber and coworkers,[10] in the details of the interaction of the chromophores with the protein, which result in tuning of the site energies. Because the time-resolved pump−probe spectrum observed in *C*-phycocyanin evolves very little following the initial line-broadening and interexciton-state relaxation dynamics, it seems likely to us that the exciton-state dynamics involving the α84−β84 chromophore dimers contribute more to directional energy transfer down the rods in the phycobilisome than is contributed by Förster energy-transfer paths between the chromophores in a given trimer on the picosecond and longer time scale.

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