

FLUORESCENCE RELAXATION KINETICS AND QUANTUM YIELD FROM THE PHYCOBILISOMES OF THE BLUE-GREEN ALGA *NOSTOC* SP. MEASURED AS A FUNCTION OF SINGLE PICOSECOND PULSE INTENSITY*

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(Received 29 October 1980; accepted 29 June 1981)

Abstract—A detailed experimental study of the effect of intensity of a 6 ps excitation pulse on the decay kinetics and yield from phycobilisomes (PBsomes) is presented. The fluorescence from the *c*-phycoerythrin (PE) emission from PBsomes was found to decay as a single exponential with a time of 31 ± 4 ps for an excitation intensity $< 10^{14}$ photons/cm² per pulse. The risetime of the *c*-phycocyanin (PC) and allophycocyanin (APC) emission from PBsomes was found to be 34 ± 13 ps. Therefore, at low excitation intensities, the energy transfer time between the constituent phycobiliproteins, PE and PC, is measured to be 34 ± 13 ps from the fluorescence decay time of PE and the fluorescence risetime of the PC and APC emission. The fluorescence yield from the PE emission component in PBsomes was found to be intensity dependent for excitation intensities $> 10^{14}$ photons/cm². The decrease in yield with increased intensity in this case occurred at a higher intensity than in the isolated phycobiliprotein PE. The fluorescence yield of the PC and APC emission component was also found to decrease markedly with increasing excitation intensity. This is in contrast to the case of the isolated phycobiliprotein APC which showed only a slight quenching of the fluorescence. The higher quenching observed for the APC emission in the PBsomes evidences the higher effective absorption of APC via energy transfer from PE to PC and APC.

INTRODUCTION

The pathway of energy transfer in the light-harvesting pigment complexes of oxygen evolving photosynthetic organisms, is most easily followed in the phycobilisomes (PBsomes)‡ (Gantt, 1975) which occur naturally in red and blue-green algae. Energy transfer in PBsomes is generally considered to occur by the Forster inductive resonance mechanism from the shorter to the longer wavelength absorbing pigments: phycoerythrin (PE) → phycocyanin (PC) → allophycocyanin (APC) (Duysens, 1952; Dale and Teale, 1970; Gantt and Lipshultz, 1973; Grabowski and Gantt, 1978; Searle *et al.*, 1978), followed by transfer to chlorophyll *a* in the thylakoid membrane. Tomita and Rabinowitch (1962) reported energy transfer times from *b*-phycoerythrin to *r*-phycocyanin of 300 ± 200 ps and from APC to chlorophyll *a* of 500 ± 200 ps. Estimates of the Forster critical distances and the interchromophore distances by Dale and Teale (1970) when combined with the intrinsic fluorescence lifetime of the bilin prosthetic group, yield a Forster pair-wise transfer time of ≤ 10 ps. More recently, by using a model of hemispherical, concentric layers for the PBsomes, Grabowski and

Gantt (1978) estimated the mean energy transfer time from the PE layer to the PC layer to be 280 ± 40 ps in the red algae. The only measurement by picosecond time-resolved fluorescence techniques so far has been made on PBsomes of *Porphyridium cruentum* by Searle *et al.* (1978), where it was found that energy transfer from *b*-PE to APC produced a rise-time for APC fluorescence of 120 ps.

The structure of the PBsomes of the blue-green alga *Nostoc* sp. (Bryant *et al.*, 1979) consists of 6 rod-like projections attached to a central hemispherical core. Each rod consists of 6 PE monomer disks stacked on top of 2 PC monomer disks. The central core consists of 12 APC monomer disks. Preliminary studies by Grabowski and Gantt (1978) have suggested that the efficiency of energy transfer is greater in PBsomes isolated from blue-green algae than from red algae. In addition, there presently exists an apparent anomaly in the fluorescence properties of APC. Measurements on isolated APC have given lifetimes of ≤ 3 ns (Grabowski and Gantt, 1978; Wong *et al.*, 1981), while it has been suggested by Searle *et al.* (1978) that the lifetime is 4 ns in isolated PBsomes of *P. cruentum*. One would expect however that the PBsomes, being an aggregate of biliproteins, should be more susceptible to exciton annihilation effects and presumably show a shorter lifetime for the APC fluorescence component than can be observed in solution. Thus, it is evident that new experimental results are necessary to

*Part III in the series.

‡Abbreviations used: APC, allophycocyanin; PBsomes, phycobilisome; PC, *c*-phycocyanin; PE, *c*-phycoerythrin.

answer some of the above questions and to gain further understanding of energy transfer in PBsomes.

The present work reports on the first application of direct picosecond fluorescence techniques to the study of PBsomes isolated from a blue-green alga, *Nostoc* sp. Fluorescence rise and decay kinetics and yields of the emission from the cyanophycean PE and combined cyanophycean PC and APC in the PBsomes were studied as a function of the intensity of a single 530 nm, 6 ps excitation pulse over the range 10^{13} – 10^{15} photons/cm².

MATERIALS AND METHODS

Nostoc sp. was grown in a fermentor on medium CG-10 (Ingram and Van Baalen, 1970) according to the method described by Rusckowski and Zilinskas (1980). Phycobilisomes were isolated as described by Zilinskas *et al.* (1978), by a modification of the procedure of Gray and Gantt (1975), and stored in pellet form at 4 °C until used. Experimental samples were prepared from pellets within a week of isolation by resuspension in 0.75 M phosphate buffer, pH 7 at 23 °C. The OD of the suspension was 0.5 at 530 nm in a 2 mm cuvette. Precautions were taken to ensure sample homogeneity and to minimize settling effects by mixing the suspension every 10 min. Absorption spectra were recorded at room temperature in a Cary 17D recording spectrophotometer. The fluorescence spectra were measured in a Aminco-Bowman spectrofluorometer equipped with R446S (Hamamatsu TV photomultiplier tube).

The fluorescence kinetics were detected using a streak camera Optical Multichannel Analyzer system previously described by Wong *et al.* (1981) and Yu *et al.* (1977). A train of ~100 pulses at 1.06 μ m was obtained from a mode-locked Nd:glass laser. A single pulse was selected by activation of a 5 ns Pockels cell shutter from a laser triggered nitrogen spark gap cell. The selected pulse was amplified and frequency doubled to provide a single excitation pulse of ~6 ps duration at 530 nm. The beam was collimated and imaged to an elliptical spot size of 2 \times 1 mm of uniform intensity onto the sample. The sample was frontally excited and the fluorescence collected with $f = 1.25$ optics and imaged onto the entrance slit of a Hamamatsu streak camera. The streak camera maps the temporal intensity profile of the fluorescence onto a spatial axis. The output streak trace is digitized by an Optical Multichannel Analyzer (OMA) from Princeton Applied Research and subsequently stored and processed in a Digital Equipment Corporation DecLab PDP 11/03 minicomputer. The data is normalized in time and intensity for non-linearities in the streak rate and is analyzed through appropriate use of signal averaging and curve fitting techniques. The time resolution of the laser-streak camera system is ≤ 12 ps.

Phycocyanin fluorescence was spectrally isolated by using a combination of a Corning 3-67 glass filter and an Optical Coating Laboratories Cyan Dichroic filter (600–800 nm cut-off). Allophycocyanin fluorescence was observed by replacing the Cyan Dichroic filter with a Magenta Dichroic filter (500–600 nm cut-off region).

RESULTS

The steady state absorption, fluorescence and excitation emission spectra of PBsomes are displayed in Fig. 1. The experimental results obtained for the transient fluorescence and quantum yield of the phycobiliprotein components in PBsomes will be presented in this section.

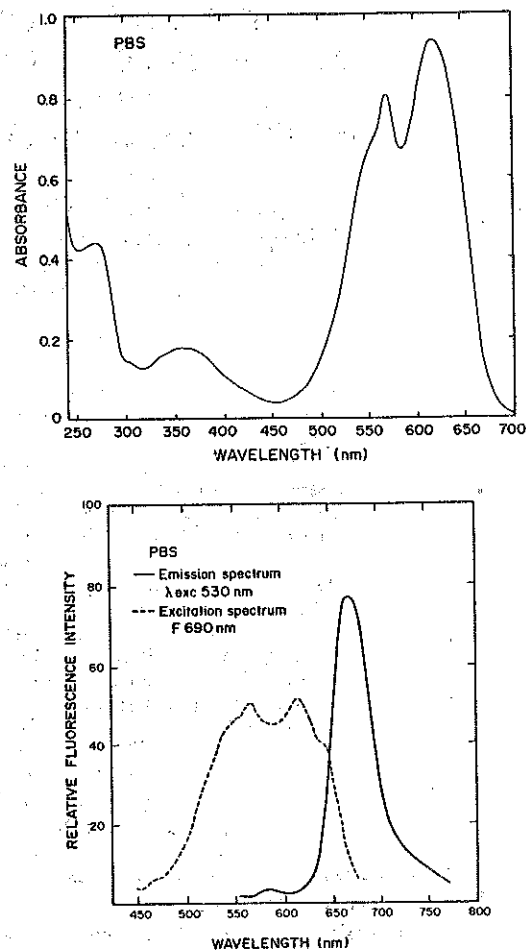


Figure 1. The absorption spectrum (a) and the fluorescence emission and excitation spectra (b) for *Nostoc* sp. PBsomes. The PBsomes were suspended in 0.75 M potassium phosphate buffer, pH 7, to an absorbance of 0.1 at 620 nm in a 1 cm pathlength cuvette for the fluorescence measurements. The fluorescence was analyzed at 690 nm for the excitation spectrum. The PBsomes were excited at 530 nm for the emission spectrum. The individual absorption maximum for biliproteins occurs at 580 nm for PE, 650 nm for PC, 660 nm for APC II and III, and 670 nm for APC I and B.

Phycocyanin fluorescence in phycobilisomes

We isolated the spectral region 560–600 nm for measurements on PE fluorescence (emission peak at 575 nm). Excitation of a suspension of PBsomes isolated from *Nostoc* sp., with a single 6 ps pulse of 530 nm showed that the rise of the PE fluorescence was within the resolution time of our instrument ≤ 12 ps. A typical fluorescence decay curve for the PE emission component in PBsomes is shown in Fig. 2. The fluorescence relaxation kinetics for PE in the PBsomes were found to be exponential and intensity independent over the excitation intensity range 10^{13} – 10^{15} photons/cm² (Fig. 3). The relaxation lifetime of PE fluorescence for intensities $\leq 10^{14}$ photons/cm² was 31 ± 4 ps, which is shorter than that reported by Searle *et al.* (1978) for PBsomes isolated

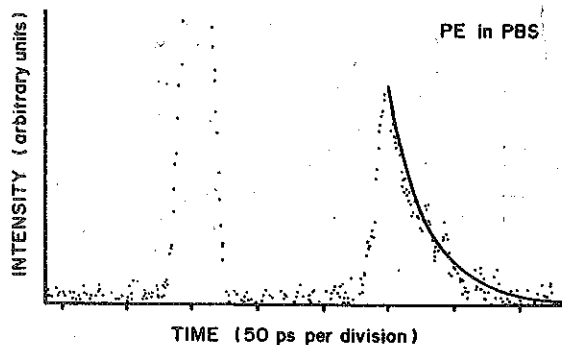


Figure 2. Emission decay profile of PE fluorescence in PBsome excited by 6 ps, 530 nm laser pulse. The dots denote the experimental trace while the solid line shows the calculated fit to the data. At an excitation intensity of 1.8×10^{14} photons/cm², the decay was fitted to a single exponential curve with a lifetime of 31 ps. The first pulse is a 530 nm prepulse used to provide a reference mark on the time axis.

from *P. cruentum*. At higher intensity the PE emission was found to decay exponentially with a lifetime of ~23 ps. This is consistent with the studies of Grabowski and Gantt (1978) who predicted a more efficient energy transfer in the PBsomes of blue-green algae than from those of the red algae. This decay time is much faster than that observed in isolated PE (Wong *et al.*, 1981) due to the presence of energy transfer to PC and APC within the PBsome.

The equation describing exciton annihilation proposed by Swenberg *et al.* (1976) was fit to the experimental data. The relative intensity scale was then normalized in order to obtain unity quantum yield at low intensity. Because of the fast relaxation decay and low quantum yield of PE due to efficient energy transfer to PC, it was not possible to extend our measurements below 5×10^{13} photons/cm² with our present apparatus. However, from similar measurements performed on other photosynthetic organisms (Wong *et al.*, 1981; Swenberg *et al.*, 1978) an excitation intensity of 5×10^{13} photons/cm² is considered low. This normalization is not completely arbitrary, since the fluor-

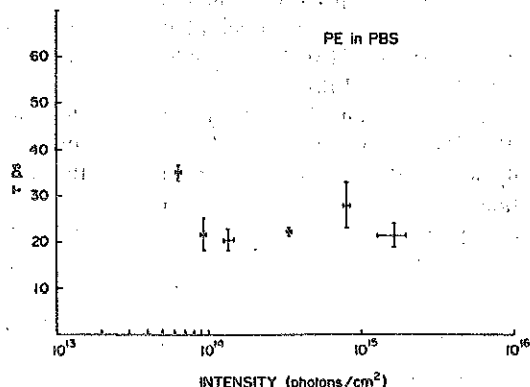


Figure 3. Plot of PE relaxation times in PBsome as a function of excitation pulse intensity.

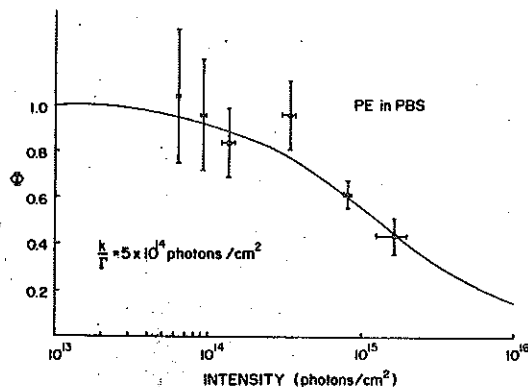


Figure 4. Relative fluorescence yield of PE in PBsome vs. excitation pulse intensity. The relative fluorescence yield was calculated as the total integrated area under the best-fit curve. Error bars denote one standard deviation.

escence quantum yield is observed to be approximately constant over the intensity region from 6×10^{13} to 3×10^{14} photons/cm² per pulse for PE. The normalized fluorescence yield is shown in Fig. 4. The fluorescence yield can be seen to decrease at an intensity of $\sim 3 \times 10^{14}$ photons/cm² per pulse. The decline of the fluorescence quantum yield from the PE emission from PBsomes occurs at a higher intensity than in the isolated phycobiliprotein PE.

Phycocyanin and allophycocyanin fluorescence

Fluorescence from PC and APC was studied by observing emission beyond 600 nm. A typical fluorescence rise curve for PC and APC emission is shown in Fig. 5 and is compared with the resolution limited risetime for a 0.2 mM solution of erythrosin in water. At low pulse intensities ($I \sim 6 \times 10^{13}$ photons/cm²), the time for the PC and APC fluorescence to rise from 10 to 90% of the maximum level was 34 ± 13 ps (14 measurements). The fluorescence decay showed a pronounced dependence on the excitation intensity (Fig. 6). Over the intensity range studied, the decay kinetics were non-exponential, but could be adequately fitted with a sum of two exponentials. The

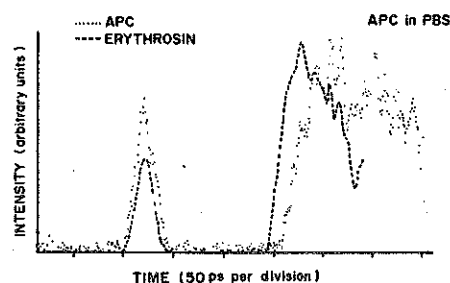


Figure 5. Fluorescence rise of APC fluorescence in PBsomes at 7×10^{13} photons/cm² per pulse, superimposed on the fluorescence rise of 0.2 mM erythrosin in water. The first pulse is a 530 nm prepulse used for time reference.

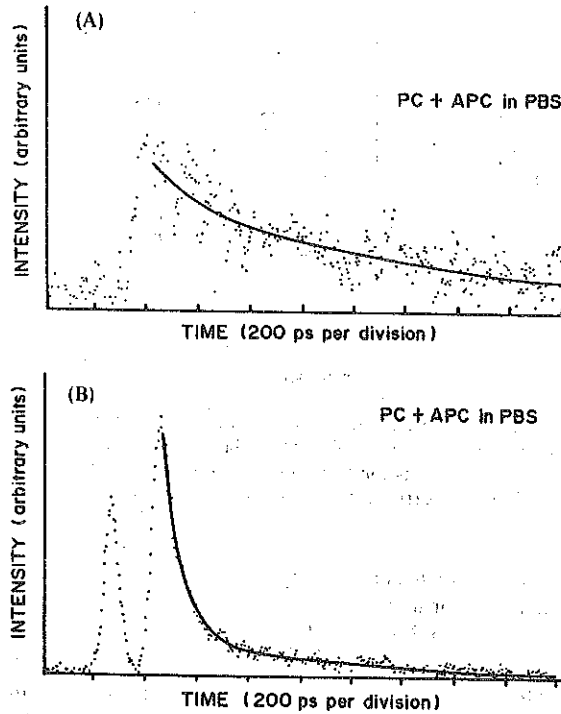


Figure 6. Decay profile of APC fluorescence in PBsomes at different single pulse excitation intensities, I . Figure A displays the fluorescence decay obtained at 2×10^{13} photons/cm² per pulse. The curve through the data is a plot of $I = e^{-t/212} + 2.1e^{-t/1377}$. Figure B displays the fluorescence decay obtained at 2.6×10^{13} photons/cm² per pulse. The curve drawn through the data is a plot of $I = 8.2e^{-t/76} + e^{-t/664}$. The first pulse in 6B is a 530 nm prepulse used for time reference.

fluorescence decay profile has been presented in terms of the equivalent decay of a single exponential in order to obtain a better understanding of the overall intensity dependence. The $1/e$ time was obtained by defining an equivalent single exponential as:

$$A \exp(-t/\tau) \equiv A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2)$$

where

$$\tau = (A_1 + A_2)\tau_1\tau_2 / (A_1\tau_2 + A_2\tau_1)$$

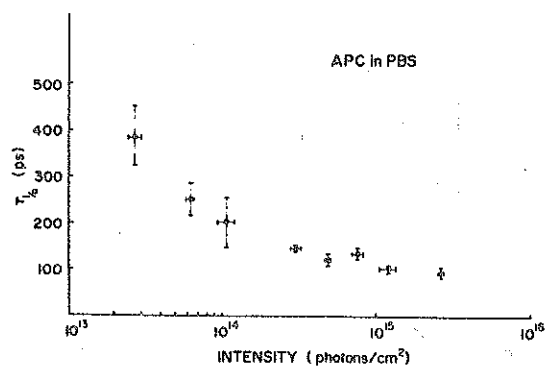


Figure 7. Plot of APC $1/e$ relaxation lifetime, vs. the excitation pulse intensity. Error bars denote one standard deviation.

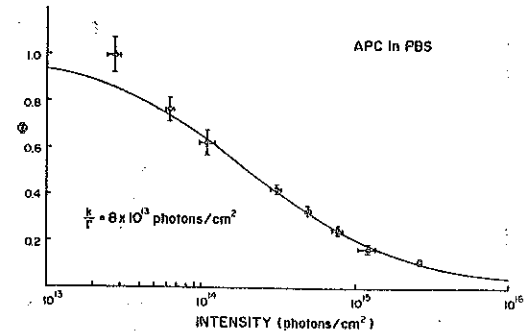


Figure 8. Relative fluorescence yield of APC vs. excitation pulse intensity. The relative fluorescence yield was based on the total integrated area under the best-fit exponential. Error bars denote one standard deviation.

In Fig. 7 a plot of the $1/e$ decay times as a function of the intensity of the excitation pulse is displayed. The relative amplitudes and lifetimes for a typical fluorescence intensity profile at both the low and high intensity regions can be described as follows: at a pulse intensity of 2.7×10^{13} photons/cm² the decay was $F = 1.2 \exp(-t/212) + \exp(-t/1174)$ and at 2.7×10^{15} photons/cm² the decay could be fitted to $F = 6.2 \exp(-t/83) + \exp(-t/716)$, where t is in picoseconds. The calculated fluorescence yield dropped by a factor of ~ 8 over the intensity range investigated (Fig. 8). This large decrease in the fluorescence quantum yield for APC in PBsomes does not follow the yield dependence observed in the isolated phycobili-protein APC, where the fluorescence quantum yield varied by less than 20% over the intensity range 10^{13} – 10^{15} photons/cm² per pulse. This is expected due to the presence of efficient energy transfer from PE to PC and APC.

DISCUSSION

The results of this investigation have shown that the fluorescence characteristics of PE in PBsomes (Fig. 2) are different from those of PE in solution (Wong *et al.*, 1981). We have found that the fluorescence decay of isolated PE in solution is exponential ($1/e$ time, 1552 ps) at pulse intensities $\leq 10^{14}$ photons/cm² and non-exponential (2 components— $1/e$ times, 130 and 1417 ps) at higher intensities (Wong *et al.*, 1981). In the PBsomes, however, the PE fluorescence relaxation lifetime at pulse intensities $\leq 10^{14}$ photons/cm² was 31 ± 4 ps (Fig. 3), and was only slightly dependent on the intensity of the excitation pulse. The decay time of PE for intensities $> 10^{14}$ photons/cm² was $\sim 23 \pm 5$ ps. The measured short-lifetime, single-component process for the relaxation of the lowest excited singlet state of PE reflects the transfer of excitation energy from PE to PC in the PBsome complex. This is supported by the measured risetime of PC and APC of 34 ps. A comparison of these findings with those of Searle *et al.* (1978) for PBsomes isolated from *P. cruentum*, indicates that the

fluorescence lifetime of PE in PBsomes is indeed short and excitation intensity independent. The nearly four-fold difference between our measurements and those of Searle *et al.* (1978) is consistent with the idea of a greater efficiency of energy transfer in PBsomes of the blue-green algae.

The decrease in the fluorescence quantum yield for the PE emission from PBsomes is comparable to that observed in the isolated phycobiliprotein PE. Since the relaxation rate k for PE in PBsomes at low intensity is ~ 30 times larger than in the isolated PE case, for the same value of the annihilation parameter, Γ , one would expect the yield to decrease at a higher intensity corresponding to a higher value of k/Γ . Our result might be explained by a more pronounced Γ due to greater singlet-singlet annihilation which can now occur not only within a single PE molecule, but among the several stacked PE molecules in the PBsome rods.

We may estimate the singlet-singlet annihilation coefficient for the case of PE in PBsomes by use of the relation for the fluorescence yield at intensity I to the fluorescence yield at low intensities:

$$\Phi(I)/\Phi(0) = \frac{k}{\Gamma I} \ln\left(1 + \frac{\Gamma I}{k}\right),$$

where k is the inverse of the lifetime of fluorescence at low intensities and Γ is an experimentally accessible parameter related to the bimolecular rate constant for singlet-singlet annihilation, γ_{ss} ($\Gamma = \gamma_{ss}\alpha/2$). From the fluorescence yield curve for PE we obtain a value of

$$\frac{k}{\Gamma_{PE/PBsome}} = 5.0 \times 10^{14} \text{ photons/cm}^2.$$

Using the experimentally measured value for k of $3.2 \times 10^{10} \text{ s}^{-1}$, allows us to calculate the singlet-singlet annihilation parameter

$$\Gamma_{PE/PBsome} = 6.4 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}.$$

This value is ~ 30 times larger than in the isolated PE molecule. The larger value for Γ_{PE} in the PBsome as opposed to that of the isolated protein can be attributed to increased contributions from both the effective local absorption coefficient and the singlet-singlet annihilation coefficient. Since PE in PBsomes occurs in unit clusters consisting of 6 phycoerythrin monomers, the local absorption coefficient is

$$\alpha_{PE/PBsome} = 6\alpha_{PE}$$

The singlet-singlet annihilation coefficient, γ_{ss} , may be higher since the closer distances for the phycoerythrobilin units in the PBsomes allow for a faster transfer of the excitation energy between the units as opposed to the case of isolated biliproteins in solution.

It is clear that the APC fluorescence risetime of 34 ps, which we have taken as the time for the fluorescence to rise from 10 to 90% of its maximum value, is in good agreement with the PE fluorescence relaxation time.

The decay of APC fluorescence from PBsomes is intensity dependent (Figs. 7 and 8), and therefore supportive of the previous interpretation (Wong *et al.*, 1981) that exciton annihilation in the PBsomes occurs at the level of the allophycocyanins. The PC and APC fluorescence emission from PBsomes can be described by a dual exponential decay. Since it is difficult to isolate the PC emission component from that of APC in PBsomes, we speculate that at low intensity ($< 10^{14}$ photons/cm²) the fast component of 189 ± 20 ps could be due to the PC emission which is indicative of energy transfer from PC to APC.

As in the case of the PE emission, we may obtain an estimate of the singlet-singlet annihilation parameter Γ_{APC} . We thus obtain from Fig. 8.

$$\frac{k}{\Gamma_{APC/PBsome}} = 8 \times 10^{13} \text{ photons/cm}^2.$$

Using the measured $k^{-1} = 1200$ ps gives

$$\Gamma_{APC/PBsome} = 1.04 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}.$$

The dramatic decrease in both the lifetime and quantum yield with intensity for the PC and APC emission component from PBsomes, as compared to the case of the isolated pigments, is the result of the efficient transfer from PE which results in a higher effective absorption coefficient for APC in PBS at 530 nm than in the isolated APC case. In PBsomes, the stacked configuration of the PE and PC units gives rise to a higher local absorption and quenching relative to the unstacked units of the pigment proteins free in solution. The radial arrangement of the phycobilin rod units in the PBsome enhances singlet-singlet annihilation processes as well as energy transfers through the natural funneling, or directed migration of excitations from PE to PC to APC. At low intensity ($< 10^{14}$ photons/cm²) both the isolated components and the PE emission from PBsomes show little quenching, $(k/\Gamma)_{APC/PBsome} \sim 8 \times 10^{13}$ photons/cm², which is ~ 100 times less than the estimated value for k/Γ for the isolated APC. This can be partly attributed to a higher effective absorption coefficient in APC since in PBsomes the relevant local absorption is that of PE. The relative local absorption of a single PE monomer is approximately 35 times larger than that of an APC monomer (Wong *et al.*, 1981). Thus, there will be a higher singlet state population for APC in PBsomes as compared to isolated APC at a given excitation intensity at 530 nm due to energy transfer. The difference between this estimated drop of ~ 35 due to the contribution from the higher effective absorption, coupled with the presence of three times more PE monomers than APC monomers in the PBsomes, can account for the observed decrease of ~ 100 in $(k/\Gamma)_{APC/PBsome}$. In addition, annihilation within and among the PE and PC units in PBsomes, as well as the cumulative transfer from the different rods to the APC core can lead to a reduction of the value of k/Γ .

CONCLUSION

Energy transfer in phycobilisomes can be directly followed through measurement of the fluorescence rise and decay kinetics of the constituent accessory pigments. The fluorescence from the PE emission from PBsomes was found to decay as a single exponential throughout the intensity region investigated with a lifetime of $\sim 31 \pm 4$ ps. The risetime of the PC and APC emission from PBsomes was found to be 34 ± 13 ps. The component fluorescence yields were found to decrease with increasing excitation intensity. This fluorescence quenching is indicative of exciton-

exciton annihilation. In particular, the dramatic fluorescence quenching observed for the APC emission in PBsomes in contrast to the lack of quenching for the isolated APC is attributed to the higher effective absorption resulting from the efficient energy transfer in the phycobilisomes.

Acknowledgements—This research was supported by National Science Foundation grant PCM 714966 and CUNY FRAP Award to R.R.A. and US Dept. of Agriculture grant 5901-0410-8-0185-0 to B.A.Z. We thank Mr. Aaron Dagen for his assistance in the computer analysis of some of the data.

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