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PICOSECOND TIME-RESOLVED ENERGY TRANSFER IN PHYCOBILISOMES ISOLATED FROM THE RED ALGA *PORPHYRIDIUM CRUENTUM* *

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Energy-transfer kinetics in isolated phycobilisomes of the red alga *Porphyridium cruentum* has been probed by picosecond absorption and fluorescence techniques upon selective excitation of the individual phycobiliproteins by a tunable picosecond laser. The fluorescence decays of B-phycoerythrin and R-phycocyanin were found to be non-exponential. It does not, however, follow an $\exp(-2At^{1/2})$ decay law. The main components in the fluorescence decays of B-phycoerythrin and R-phycocyanin have lifetimes of approx. 60 and approx. 40 ps, respectively, as a result of energy transfer. In addition a second decay component with small relative amplitude is required for a good description of the energy-transfer kinetics. This component has a lifetime in the range of approx. 200 ps (B-phycoerythrin) and 550 ps (R-phycocyanin). The fluorescence decay in the main emission band is non-uniform with two components of 1.0 and 1.8 ns. The energy transfer processes were found to occur sequentially from B-phycoerythrin to R-phycocyanin and allophycocyanin, in agreement with an earlier study (Searle, G.F.W., Barber, J., Porter, G. and Tredwell, C.J. (1978) Biochim. Biophys. Acta 501, 246–256). Measurements of transient absorption anisotropy revealed the presence of two processes leading to fast depolarization in B-phycoerythrin. The anisotropy decay times have values of 12 and 150 ps. The shorter one is attributed to intramolecular energy transfer within B-phycoerythrin monomers. the longer one either arises from transfer within the B-phycoerythrin pigment bed or is related to the transfer from B-phycoerythrin to R-phycocyanin. Rising-terms are observed in the fluorescence kinetics of the indirectly excited pigments. Their rate constants agree well with those determined from the decay of the directly excited pigments. The characteristic energy-transfer time from B-phycoerythrin at the periphery to the terminal emitter is around 70 ps. This fast transfer ensures an efficiency of better than 98%. The overall energy-transfer kinetics in these hemiellipsoidal phycobilisomes is found to be very similar to that found in hemidiscoidal phycobilisomes.

Introduction

Both blue-green algae (Cyanobacteria) and red algae (Rhodophyceae) contain different types of water-soluble phycobiliproteins which are aggregated to morphologically distinct supramolecular complexes, the so-called phycobilisomes. These serve as light harvesting pigments of the photosynthetic apparatus and transfer absorbed light energy preferentially to chlorophyll *a* of Photosystem II. Intact phycobilisomes may be detached from the photosynthetic membrane by means of detergents. Their native structure is retained in high-molarity aqueous buffer solution [1,2]. Phycobilisomes from Rhodophyceae have been first isolated

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by Gantt et al. [3] from *P. cruentum* and by Koller et al. [4] from *Rhodella violacea*. Isolated intact phycobilisomes exhibit a main fluorescence band in the range 670–680 nm which is attributable to allophycocyanin and/or to coloured linker polypeptides [5]. The fine structure [6,7] and the polypeptide composition [8,9] of *P. cruentum* phycobilisomes have been described as well. They consist of B-phycoerythrin, R-phycocyanin, allophycocyanin, and linker peptides.

Time-resolved measurements of the excited state kinetics in the picosecond range can give detailed information about the mechanism of energy transfer in such antenna pigments. The first picosecond fluorescence studies have been undertaken by Porter and coworkers on intact algae of *P. cruentum* [10] and on isolated phycobilisomes [11]. The authors introduced the $\exp(-2At^{1/2})$ kinetics into the analysis of the energy transfer kinetics. More recently, the energy transfer in phycobilisomes from the cyanobacteria *Nostoc sp.* [12] and *Mastigocladus laminosus* [13] has been examined by picosecond methods. Time-resolved fluorescence spectra of he phycobiliproteins in intact algae have been reported very recently by Mimuro et al. [14]. The first picosecond study of phycobilisomes with selective excitation of the individual pigments by means of a tunable laser and applying both fluorescence and transient absorption techniques has been carried out by ourselves [15] on phycobilisomes of *R. violacea*. We had found that the energy-transfer kinetics does not follow a single exponential decay. Furthermore, the phenomenon of transient anisotropy related to energy transfer in phycobilisomes has been dealt with very recently by Hefferle et al. [13] and by Gillbro et al. [16].

A general problem encountered in the study of photosynthetic antenna systems is the occurrence of singlet-singlet exciton annihilation already at modest excitation intensities [12,17,18]. One therefore prefers to use fairly low-intensity excitation pulses. Besides the energy-transfer kinetics between the different phycobiliproteins in phycobilisomes, the question of possible intramolecular energy transfer (homotransfer) within an individual phycobiliprotein, which is part of the antenna complex, is a challenging problem as well. A time-resolved fluorescence kinetic study proving the occurrence of homotransfer within an isolated phycobiliprotein has been carried out very recently by ourselves [19].

The aims of the present work are the following: Firstly, to extend – along the above guidelines – the work of Searle et al. [11] on phycobilisomes of *P. cruentum*. These phycobilisomes are of the hemiellipsoidal type in contrast to the hemidiscoidal phycobilisomes of *R. violacea* [20] which we have studied earlier [15]. Secondly, to excite and detect selectively the individual phycobiliproteins in order to resolve also the kinetics of R-phycocyanin, which has not been achieved previously [11]. Thirdly, to investigate the anisotropy decay within the B-phycoerythrin and R-phycocyanin pigment bends, which should reveal further details on the energetic coupling of the various chromophores present in phycobilisomes. Measurements of transient anisotropy are ideally suited to answer this question. Fourthly, to reexamine the unusually long APC decay time (approx. 4 ns) that had been reported earlier for *P. cruentum* phycobilisomes [11]. Finally, to carry out the above experiments with excitation pulses of low photon density in order to rule out any possibility of singlet-singlet annihilation effects.

Since the phycobilisomes of *P. cruentum* are of hemiellipsoidal type, comparison of our data with those obtained on other phycobilisomes and with model calculations [21] should reveal possible similarities or differences in the energy transfer function brought about by these structural differences. So far, also the question regarding the exact kinetics of energy transfer in such antenna systems, i.e., sum of exponentials, $\exp(-2At^{1/2})$, vs. some other type of non-exponential kinetics, is not yet resolved [10,15]. In our earlier study a decision had not yet been possible basically because of the presence of approx. 10% dissociated phycobilisomes which gave rise to a long-lived fluorescence background [15]. Phycobilisomes of *P. cruentum* are especially suited to solve this problem since they represent the most stable phycobilisomes known to us so far. A method with excellent signal-to-noise ratio is prerequisite to resolve this question.

Materials and Methods

Porphyridium cruentum (syn. = *Porphyridium purpureum*) was grown in homocontinuous mass culture at 20°C in artificial seawater medium [22] aerated with 2% CO_2 in 98% air under a 16 : 8 h light-dark regime. For phycobilisomes isolation 10–15-day old cells were harvested by low speed centrifugation, washed twice in 0.75 M potassium phosphate puffer (pH 7.0) and disrupted by repeated ultrasonic treatment in an ice-cooled steel chamber with a Branson Sonifier Mod. S 125 (5×30 s at output power level 5). The homogenate was incubated in 1% (v/v) Triton X-100 at 20°C for 15 min. under continuous stirring. The suspension was purified by centrifugation for 20 min. at $48\,000 \times g$ and 15°C. 2 ml Aliquots of the supernatant were layered on continuous sucrose gradients (0.2–1.0 M in 0.75 M potassium phosphate buffer (pH 7.0), 32 ml per centrifuge tube). After 3.5 h centrifugation at $80\,000 \times g$ in a preparative ultracentrifuge (TGA 65, Kontron, Munich) the phycobilisomes bands were pooled and enriched by ultrafiltration in an Amicon cell using XM 100 filters. The clear non-scattering phycobilisomes suspension was stable for more than 2 weeks when stored at 18–20°C, as judged by the constancy of its fluorescence parameters.

Steady-state fluorescence measurements were performed on a computer-controlled Spex Fluorolog instrument [23]. For emission spectra the bandwidth was 5 nm in the excitation monochromator and 2.5 nm in the emission monochromator and vice versa for excitation spectra. Emission and excitation spectra of the highly concentrated samples were measured in 1 mm cuvettes in order to avoid self-absorption and other nonlinear effects. Maxima are accurate to ± 2 nm. All spectra presented were corrected for wavelength dependence of photomultiplier sensitivity and lamp intensity. Absorption spectra were measured on a Perkin Elmer Model 320 ultraviolet/VIS spectrometer in a 2 mm cuvette.

Time-resolved fluorescence measurements were carried out using a synchronously pumped modelocked and cavity-dumped dye laser system (Spectra Physics) with an argon-ion laser as the pumping source. This system provides pulses of 5–15 ps duration (full width at half maximum) with variable repetition rate. The dye laser was operated with either rhodamine 6G (tuning range, 570–650 nm) or rhodamine 110 (tuning range, 540–600 nm). The detection system was a single-photon timing apparatus capable of measuring lifetimes below 20 ps [15,19,24]. Fluorescence was selected by a double-monochromator with slits set to give a 4 nm bandwidth. The width of the exciting pulse, as measured by single-photon timing, was approx. 200 (full width at half maximum) and 460 ps (full width at tenth maximum). Some measurements were carried out with an improved set-up having an apparatus function of 140 ps full width at half maximum. The high sensitivity of the single-photon timing technique enabled us to use laser pulse intensities for excitation in the range $1 \cdot 10^{10}$–$1 \cdot 10^{11}$ photons/cm^2, working at a repetition rate of 400 or 800 kHz. Between 20 000 and 50 000 counts were collected in the peak channel. Fluorescence was excited with the vertically polarized laser beam and detected through a sheet polarizer set to the magic angle of 54.7° for measuring the isotropic decay function. Lifetimes according to multiexponential kinetics and/or a combination of purely exponential and $\exp(-2At^{1/2})$ kinetics were calculated by an iterative convolution method. The quality of fits was judged by both a reduced χ^2 criterion and a plot of weighted residuals [25]. The necessary requirements allowing to carry out decay analyses in terms of three of four exponentials are discussed in part in Ref. 24 and will be fully described in the forthcoming publication (Wendler and Holzwarth, in preparation). All measurements were carried out at room temperature (23–25°C) in 0.75 M phosphate buffer (pH 7.0) in air-saturated solution.

The basic apparatus used for the transient absorption (pump probe) measurements [15] has been supplemented with a Fresnel rhomb in the excitation beam and a Glan-Thompson polarizer in the probe beam. The Fresnel rhomb allowed to rotate the excitation polarization direction. Thus pump probe measurements could be performed with different relative polarization of excitation and probe beam, i.e., parallel, perpendicular or at magic angle to determine the transient absorption anisotropy $r(t)$. Pulse intensities for the pump probe measurements were in the range $5 \cdot 10^{13}$ to $1 \cdot 10^{14}$ photons/cm^2. No singlet-singlet annihilation ef-

fects have been observed in this intensity range within the B-phycoerythrin absorption band. This is in line with similar measurements on *Anacystis nidulans* [16]. Data evaluation of the pump probe measurements has been carried out by digitizing the recorded data followed by computer fitting according to the least-squares principle.

Results

Steady-state fluorescence and absorption

Fig. 1 shows the absorption (A), fluorescence and fluorescence excitation spectra (B) of a solution of *P. cruentum* phycobilisomes in phosphate buffer. There is good agreement between the absorption and excitation spectra which are independent of the emission wavelength indicating the high purity and integrity of the phycobilisomes preparation under study. Table I summarizes the band maxima of the above spectra. The relative intensity of the 578 nm emission band is approx. 5% of the main emission band at 676 nm.

Time-resolved fluorescence of directly excited pigments

The different constituent pigments of phycobilisomes from *P. cruentum* were excited near their respective absorption maxima at 543 and 567 nm (B-phycoerythrin), 620 nm (R-phycocyanin) and 645 nm (allophycocyanin). Fluorescence kinetics of these excited pigments were detected at 555, 570 and 580 nm (B-phycoerythrin), 640 nm (R-phycocyanin), 660, 675 and 685 nm (allophycocyanin), respectively.

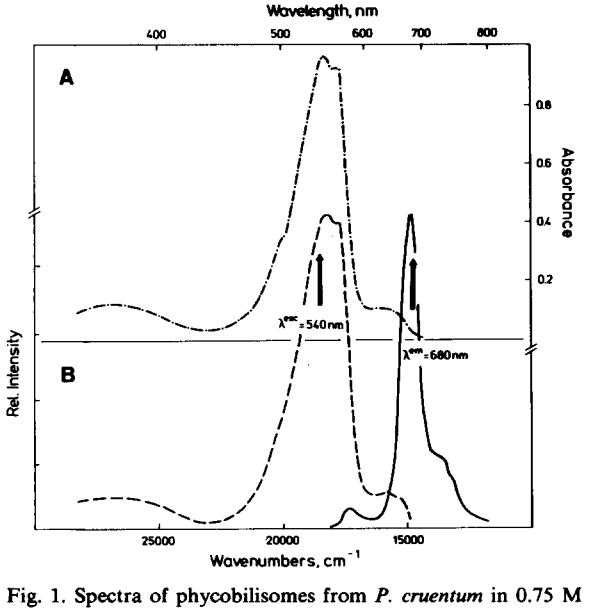

Fig. 1. Spectra of phycobilisomes from *P. cruentum* in 0.75 M phosphate buffer (pH = 7.0). (A) Absorption spectrum $d = 1$ mm; dash-dotted), and (B) corrected fluorescence (full line) and fluorescence excitation (dashed) (cf. Table I).

TABLE I

MAXIMA OF THE ABSORPTION, FLUORESCENCE AND FLUORESCENCE EXCITATION SPECTRA OF PHYCOBILISOMES FROM *P. CRUENTUM* IN 0.75 M PHOSPHATE BUFFER

(pH = 7.0) sh., shoulder.

λ_x^{abs} [a] (nm)	λ_{max}^{exc} [b] (nm)	λ_{max}^{em} [b] (nm)
493 (sh.)	495 (sh.)	–
545	547	–
563	565	578
623	625	673
650 (sh.)	650 (sh.)	730 (sh.)

[a] Accuracy of wavelength, ±1 nm.
[b] Accuracy of wavelength, ±2 nm.

The measured fluorescence decay curves were either fitted with a sum of just purely exponentials or a sum of both $\exp(-2At^{1/2})$ and exponential functions. All results obtained for a pure exponential analysis are summarized in Table II. Both B-phycoerythrin and R-phycocyanin show very short-lived decay components in the picosecond range and some contribution of a longer lived decay in the nanosecond range. Typical examples of the decay functions are give in Fig. 2A and B for B-phycoerythrin and R-phycocyanin, respectively. A fit of two exponential functions, one for the short-lived and one long-lived component results in a poor χ^2 value (larger than 1.4) and a strong systematic deviation of the residuals. However, excellent fits with χ^2 values generally below 1.1 and basically without indication of correlation of the residuals were obtained when the decays were fitted with a sum of three exponential functions. Most of the fluorescence decays with a lifetime of approx. 60 ps (average value) for B-phycoerythrin and approx. 40 ps for R-phycocyanin (cf. Fig. 2A and B and Table II). When B-phycoerythrin was excited at 543 nm the

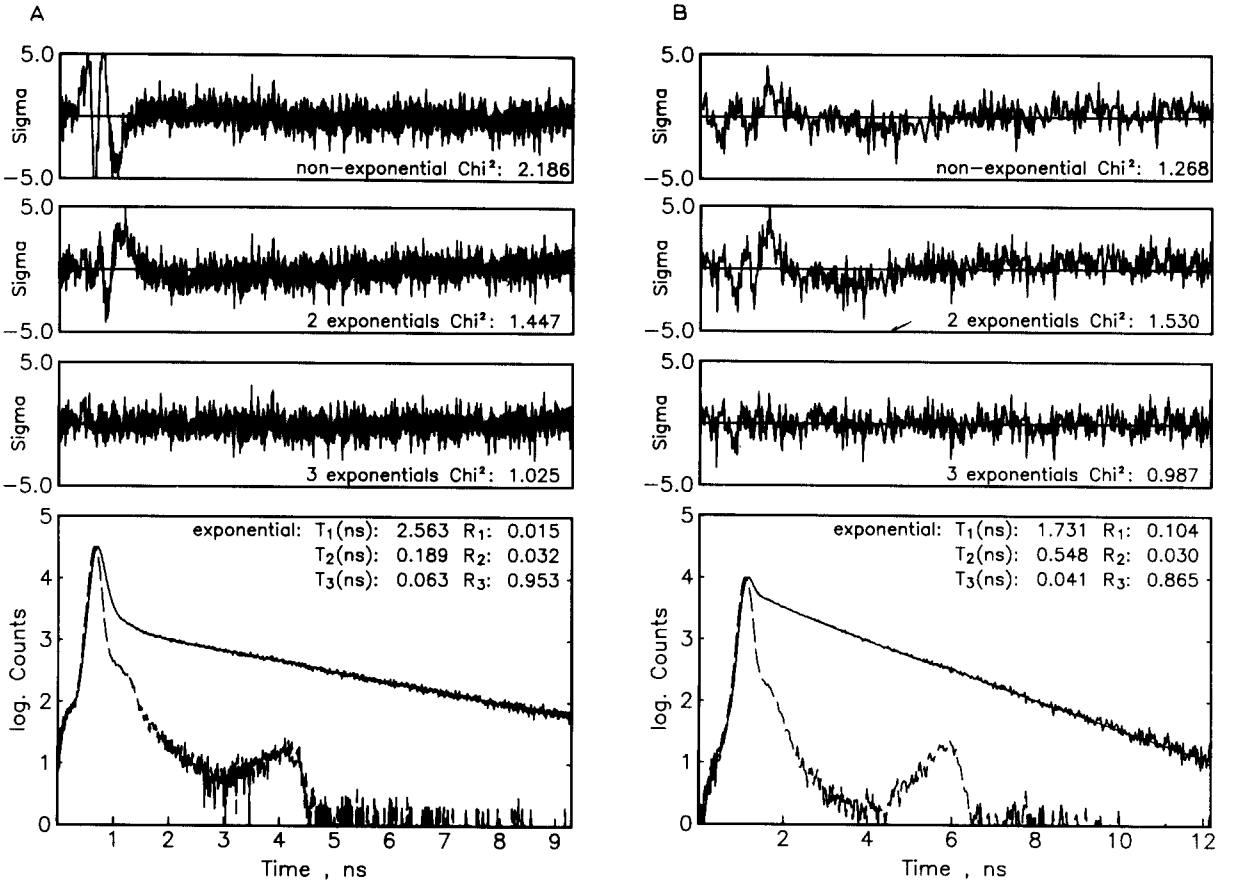

Fig. 2(A). Semilogarithmic plot of the isotropic fluorescence decay of phycobilisomes from *P. cruentum* detected at 580 nm (B-phycoerythrin); $\lambda^{exc} = 567$ nm. The frame on top gives the residuals plot for a sum of an $\exp(-2At^{1/2})$ decay and an exponential function for the nanosecond component ('non-exponential'). (B). Semilogarithmic plot of isotropic fluorescence decay of phycobilisomes from *P. cruentum* detected at 640 nm (R-phycocyanin); $\lambda^{exc} = 620$ nm. The frame on top gives the residuals plot for a sum of an $\exp(-2At^{1/2})$ decay and an exponential function for the nanosecond component ('non-exponential').

decays are slightly faster, giving an average value of approx. 50 ps. A fit involving an $\exp(-2At^{1/2})$ function for the short-lived component(s) and purely exponential function for the long-lived component resulted in a significantly poorer fit as compared to the triexponential model (cf. residual plot, top in Fig. 2A).

When allophycocyanin was excited directly, a triexponential fit was required at $\lambda_{em} = 660$ nm. In contrast a biexponential fit with lifetimes of both components in the ns range was sufficient for $\lambda_{em} = 670$ nm. The third short decay component (110 ps) at $\lambda_{em} = 660$ nm had a considerably smaller relative amplitude as compared to the B-phycoerythrin and R-phycocyanin decays (cf. Table II).

Time-resolved fluorescence of indirectly excited pigments

The results of the measurements on the indirectly excited pigments are also listed in Table II. Fig. 3 shows a semilogarithmic plot of the R-phycocyanin fluorescence detected at 640 nm upon excitation of B-phycoerythrin at 567 nm. A similar situation was found when B-phycoerythrin was excited at 543 nm and the fluorescence from R-phycocyanin was detected at 640 nm again. When fitted with exponential functions a sum of at least four exponentials was necessary to give a reasonable fit. One of them has a negative amplitude describing an indirect population (rising term) of the detected excited state(s). The necessity of at least four exponentials is expected on the basis of

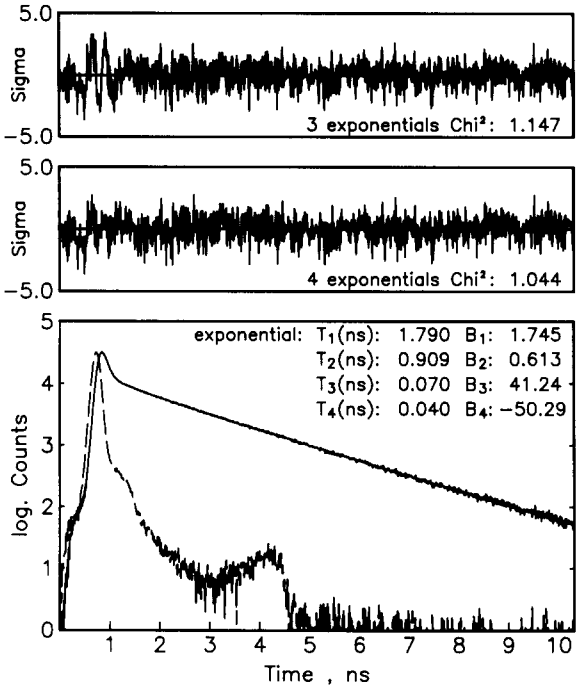

Fig. 3. Semilogarithmic plot of the fluorescence kinetics of indirectly excited R-phycocyanin in phycobilisomes from *P. cruentum* detected at 640 nm; $\lambda^{exc} = 567$ nm. The $B_1 \ldots B_4$ values in the inset are absolute coefficients. A negative sign indicates a risetime. Note for Figs 2 and 3. Superimposed on the experimental decay is the fluorescence (thin line) calculated from the best fit parameters (cf. inset). Also shown is the semilogarithmic presentation of the exciting pulse (dashed). In the inset are given the lifetimes ($\tau_1 \ldots \tau_n$) and relative amplitudes ($R_1 \ldots R_n$) of the exponential decay components. The frames above the main figure show the weighted residuals plot obtained for different kinetic model functions. The residuals indicate the deviations between the computer-fitted and experimental decay curves. Also shown in the residuals plots are the reduced χ^2-values from the statistical analysis.

the data from the directly excited pigments. It should be realized that the presence of a kinetic component contributing only a few percent to the relative fluorescence yield (product of lifetime and relative amplitude) cannot in general be recognized on the basis of the χ^2 criterion alone. This situation arises with very short decay components and especially with short rising terms. Inspection of the weighted-residuals plot in the vicinity of the maximum of the fluorescence decay curve and in the rising part provides a better criterion to decide upon the correct kinetics. According to this criterion the four-exponential fit yielding a rising term is indeed strongly favoured as compared to the three-exponential one.

When excited near the absorption maxima of B-phycoerythrin and R-phycocyanin at 567 nm and 620 nm, respectively, the fluorescence decay curves recorded in the range of the allophycocyanin fluorescence $\lambda_{em} = 660$–685 nm) clearly show a rising term when fitted with a sum of three exponential functions. However, as judged by the χ^2-criterion and the deviation plot, a sum of four exponentials is necessary to obtain acceptable fits (cf. Table II).

Transient anisotropy measurements by the pump probe technique

Fig. 4 shows the results of a pump probe measurement at $\lambda_D = 563$ nm (B-phycoerythrin) with parallel ($I_{\parallel}(t)$) and perpendicular ($I_{\perp}(t)$) polarization of the pump and probe beams. The anisotropy decay function is given theoretically by:

$$r(t) = \frac{I_{\parallel}(t) - I_{\perp}(t)}{I_{\parallel}(t) + 2I_{\perp}(t)} = \frac{D(t)}{S(t)} \quad (1)$$

This $r(t)$ function cannot be evaluated directly in

TABLE II

LIFETIMES AND AMPLITUDES OF THE FLUORESCENCE DECAYS OF PHYCOBILISOMES FROM *P. CRUENTUM* IN 0.75 M PHOSPHATE BUFFER (pH = 7.0), AS A FUNCTION OF EXCITATION AND EMISSION WAVELENGTHS

Decay fitted with a sum of exponential functions. The lifetimes are given in picoseconds; the values in parentheses are normalized relative coefficients in percentage; – denotes the rising term. The errors in lifetimes are ±10% for the picosecond components and ±5% for the nanosecond components; + denotes that absolute amplitudes are given in parentheses.

λ^{exc}	λ^{em}						
	555 nm	570 nm	580 nm	640 nm	660 nm	670 nm	685 nm
543 nm	52 (95)	42 (78)	44 (89)	36 (−50) +			
	220 (3)	100 (20)	200 (9)	53 (+43) +			
	2440 (2)	2590 (2)	2590 (2)	850 (+0.4) +			
	–	–	–	1890 (+1.0) +			
567 nm	–	–	63 (95)	63 (−5.9) +			
	–	–	190 (3)	44 (+6.3) +			
	–	–	2560 (1.5)	1060 (+0.07) +			
	–	–	–	1840 (+0.14) +			
620 nm	–			41 (87)		43 (−16) +	44 (−10) +
	–			550 (3)		49 (+12) +	190 (+0.8) +
	–			1730 (10)		980 (+0.6) +	1180 (+3) +
				–		1800 (+2) +	1890 (+4) +
645 nm	–			–	110 (17)	940 (26)	940 (25)
	–			–	1100 (29)	1840 (74)	1810 (75)
	–			–	1840 (54)		

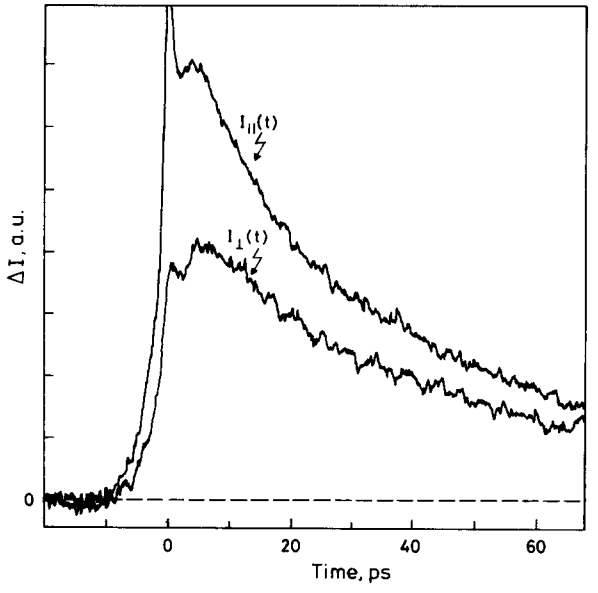

Fig. 4. Transient absorption signals of phycobilisomes from *P. cruentum* measured by the pump probe technique. $I_{\parallel}(t)$ signal observed with parallel polarization of the pump- and probe beam; $I_{\perp}(t)$ vertical polarization.

our case, since it contains contributions from two different origins. We thus calculated the difference function (anisotropic decay) $D(t)$ and the sum

TABLE III

ISOTROPIC AND ANISOTROPY DECAY DATA OF PHYCOBILISOMES FROM *P. CRUENTUM* DERIVED FROM THE PUMP PROBE MEASUREMENTS AT λ_D = 567 nm

$D(t)$ and $S(t)$ are the nominator and denominator of Eqn. 1, respectively. r_A and r_B are the anisotropy decay components (Eqn. 2).

	Lifetimes		Amplitudes	
	τ_1 [a] (ps)	τ_2 [b] (ps)	A_1	A_2
$D(t)$	6	42	86.5	55.7
$S(t)$	12	59	210	375
$r_A(t)$	12 ± 5	–	0.41	–
$r_B(t)$	150 ± 50	–	0.15	–

[a] Error ±10% for the long-lived $D(t)$ and $S(t)$ components.
[b] Error ±20% for the short-lived $D(t)$ and $S(t)$ components.

function (isotropic decay) $S(t)$ independently. A computerfit to each of these functions required a sum of two exponentials. These data are given in Table III.

In case of two decay components A and B, respectively, contributing to the isotropic and anisotropic decays the anisotropy function $r(t)$ can be written as:

$$r(t) = \frac{r_A(t)S_A(t) + r_B(t)S_B(t)}{S_A(t) + S_B(t)} = \frac{D(t)}{S(t)} \quad . \qquad (2)$$

where $r_A(t)$ and $r_B(t)$ represent the time-dependent anisotropy of components A and B, respectively, while the functions $S_A(t)$ and $S_B(t)$ are the corresponding isotropic decays. These functions are related to the parallel and perpendicular components via $D(t)$ and $S(t)$ (cf. Eqn. 1). Assuming single exponential functions for both $r_A(t)$ and $r_B(t)$, the products in the nominator of Eqn. 2 are represented by exponential functions, and the anisotropy decay times of the two components A and B can be calculated. They are found to be 12 and 150 ps, respectively. Another measurement carried out with lower time resolution over a longer time range than the one given in Fig. 4 confirmed these results. It was basically single exponential revealing only the longer of the two decay components.

Unfortunately, pump probe measurement at the R-phycocyanin absorption maximum around 620 nm were not possible because of the low absorption at this wavelength.

Discussion

From the results of the time-dependent fluorescence measurements compiled in Table II it can be concluded that the decay kinetics of the directly excited pigments at all wavelengths can be well described by a sum of three exponential components. The main components of the fluorescence of B-phycoerythrin and R-phycocyanin possess a relative amplitude of approx. 90% and decay with lifetimes of 60 and 40 ps, respectively. The slightly faster decay of approx. 50 ps for B-phycoerythrin when excited at 543 nm might possibly be due to an additional fast and unresolved component arising from an intramolecular transfer between phycoerythrin chromophores. This is in line with the fast (12 ps) component from the pump probe measurement. The intermediate decay components with a small relative amplitude and with lifetimes of about 100–200 ps (B-phycoerythrin) and 550 ps (R-phycocyanin) are not so well defined. We believe that these intermediate components reflect the actual deviation from strict exponential kinetics for energy transfer. Thus, the fast and the middle components together describe the energy transfer kinetics in the intact phycobilisomes. The origin of the long-lived component is discussed below. While the main transfer time for B-phycoerythrin agrees well with literature data [10,11], we find a considerably shorter decay of directly excited R-phycocyanin in phycobilisomes than that reported for the intact alga [10]. It is impossible to fit our data with an $\exp(-2At^{1/2})$ function alone. Even a sum of an $\exp(-2At^{1/2})$ plus an exponential function does not fit well. With such a combination of functions a value of 0.295 $ps^{-1/2}$ is obtained for the non-exponential rate constant $2A$ for B-phycoerythrin, in good agreement with the value of 0.26 $ps^{-1/2}$ reported by Porter et al. [10] and Searle et al. [11]. The corresponding value for R-phycocyanin is 0.35 $ps^{-1/2}$. The residual plots, however, show very strong systematic variations for this model (cf. Fig. 2A and B, top). Thus the $\exp(-At^{1/2})$ decay law can be excluded as a valid description of the transfer kinetics in phycobilisomes.

It had already been pointed out [15] that the assumptions made for the derivation of the $\exp(-t^{1/2})$ kinetics [26] are probably not justified for aggregated phycobiliproteins and phycobilisomes. The most critical ones are the following: (a) random orientation of donors and acceptors; (b) low donor concentration in order to exclude donor-donor interactions and (c) high acceptor concentrations as compared to donor concentration. Nevertheless, there is some degree of non-exponentiality present in the decay kinetics. This non-exponentiality can be well approximated numerically by a sum of two exponential functions. This does not necessarily imply that the real transfer kinetics does follow such a double-exponential decay.

The pump probe measurements reveal the presence of a biexponential decay of the absorption

anisotropy of B-phycoerythrin with decay constants of 12 and 150 ps. The faster one is attributed to an intramolecular energy-transfer process, presumably from so-called sensitizing (s) to fluorescing (f) chromophores [27] within a B-phycoerythrin monomeric unit. Depending on the relative orientation of these chromophores a single transfer step is sufficient to bring about a large depolarization. Our interpretation is supported by the fact that the isotropic and the anisotropic components have identical decay times (12 ps). The longer one of the isotropic lifetimes of the pump probe measurements agrees well with the short lifetime obtained from the isotropic fluorescence decay. The anisotropy decay time (150 ps) of this component is considerably longer than the isotropic one. Basically there exist two possibilities. Either the anisotropy decay of 150 ps and the isotropic lifetime of 60 ps (pump probe measurement) are related to the same process, i.e., energy transfer from B-phycoerythrin to R-phycocyanin. The other, more likely, possibility would imply a phycoerythrin-phycocyanin transfer time of 60 ps (isotropic component) in good agreement with the fluorescence decay data (60–65 ps main component), while the anisotropy decay of 150 ps could arise from energy-transfer processes occurring with the B-phycoerythrin dodecameric units between f-chromophores with different orientations of their transition moments. Comparison of the isotropic and anisotropic decay times lead us to conclude that such types of transfer processes are less likely to occur than those between f-chromophores of parallel orientation. With regard to the anisotropy decays the situation found here is very similar to that reported for the C-phycocyanin rods of *Anacystis* phycobilisomes [16]. The longer one of our anisotropy decays is of the same order as that reported by Hefferle et al. [13]. However, a second short component was not observed in their measurements.

In the case of the indirectly excited pigments at least four kinetic components including a rising term (negative amplitude) are required for a good fit of the fluorescence decay. The good agreement between the kinetic constants for the decays and the rising terms proves the sequential transfer of excitation energy in the sequence B-phycoerythrin → R-phycocyanin → allophycocyanin, in agreement with Searle et al. [11]. The decay kinetics of R-phycocyanin had not been resolved in their measurement, however.

The long-lived components (2.58 ns at λ_{em} = 580 nm and 1.73 ns at λ_{em} = 640 nm) are attributed to a small amount of partially dissociated phycobilisomes. These are energetically decoupled particles with B-phycoerythrin and R-phycocyanin as terminal emitters. A good measure for their concentration is provided by the relative amplitude of these long-lived decay components (1–2% for B-phycoerythrin and approx. 10% for R-phycocyanin). This is in agreement with our earlier findings for phycobilisomes of *Rhodella violacea* [15]. The content of such particles is, however, smaller in phycobilisomes of *Porphyridium*. Again their percentage is slightly variable for different preparations in contrast to their lifetimes. Their lifetimes are very similar to those found in phycobilisomes of *Rhodella* [15]. We believe that these long lifetimes are very close to or even identical with those of the free phycobiliproteins. In phycobilisomes of both *Porphyridium* and *Rhodella* the phycocyanin-allophycocyanin attachment seems to be the weakest link. The main decay component for allophycocyanin has a lifetime of 1.84 ns in contrast to the data (approx. 4 ns) of Ref. 11, but is in full agreement with our results for phycobilisomes of *R. violacea* [15] and of *A. nidulans* (Synechococcus 6301) (Suter, G.W., Mazzola, P., Wendler, J. and Holzwarth, A.R., unpublished results). We could not find such a long decay component. A possible explanation for the appearance of the 4 ns lifetime [11] in the main emission band of the phycobilisomes might be the presence of some chlorophyll *a*. This could have been either attached to the phycobilisomes or present as a free pigment. The first possibility would appear to be more likely since their allophycocyanin rise time of 120 ps upon B-phycoerythrin excitation [11] contrasts with our value of approx. 70 ps. The longer risetime in their case would be consistent with an additional transfer step to chlorophyll.

In the range of the allophycocyanin emission band (λ_{em} = 660–685 nm) the fluorescence does not decay with a uniform time constant. Rather, in addition to the main 1.84 ns decay component about 25% of the excited states decay with a

lifetime of approx. 1.0 ns. The amplitude of this component is prominent enough to be attributed to a major constituent of phycobilisomes. It is interesting to speculate whether this additional 1.0 ns decay component (average value) found in the range $\lambda_{em} = 660-685$ nm might in fact be related to the presence of the coloured polypeptide of 95 kDa [5]. It seems possible that some of the phycobilisomes carry this linker peptide as the terminal chromophore, while the majority does not as a result of a strong binding of this protein to the membrane or a loss during the phycobilisomes isolation procedure, Redlinger and Gantt [5] also found this polypeptide to be present both in isolated phycobilisomes and in thylakoids.

In general our data are in reasonable agreement with those reported in Refs. 10 and 11. The existing differences may be explained by the difference in sample preparation and by the higher dynamic range and larger time resolution of the single-photon timing technique used in this work.

Our results on the indirectly excited fluorescence of the phycobiliprotein confirm the sequential energy transfer phycoerythrin → phycocyanin → allophycocyanin [11]. The measured main energy-transfer times of 60 ps (phycoerythrin → phycocyanin) and 40 ps (phycocyanin → allophycocyanin) are considerably faster than those derived from a theoretical three layer model [21]. Comparing the overall results given in this work with those obtained from hemidiscoidal *Rhodella* phycobilisomes [15] we conclude that, despite of the different morphology of the hemidiscoidal and the hemiellipsoidal type of phycobilisomes, the energy-transfer properties are similar. This may be due to a similar substructural organization of the phycobilisomes, at least of the peripheral rods.

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