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# PICOSECOND TIME-RESOLVED ENERGY TRANSFER IN *PORPHYRIDIUM CRUENTUM*

## PART I. IN THE INTACT ALGA

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## Summary

The wavelength-resolved fluorescence emission kinetics of the accessory pigments and chlorophyll *a* in *Porphyridium cruentum* have been studied by picosecond laser spectroscopy. Direct excitation of the pigment B-phycoerythrin with a 530 nm, 6 ps pulse produced fluorescence emission from all of the pigments as a result of energy transfer between the pigments to the reaction centre of Photosystem II. The emission from B-phycoerythrin at 576 nm follows a nonexponential decay law with a mean fluorescence lifetime of 70 ps, whereas the fluorescence from R-phycocyanin (640 nm), allophycocyanin (660 nm) and chlorophyll *a* (685 nm) all appeared to follow an exponential decay law with lifetimes of 90 ps, 118 ps and 175 ps respectively. Upon closure of the Photosystem II reaction centres with 3-(3,4-dichlorophenyl)-1,1-dimethylurea and preillumination the chlorophyll *a* decay became non-exponential, having a long component with an apparent lifetime of 840 ps. The fluorescence from the latter three pigments all showed finite risetimes to the maximum emission intensity of 12 ps for R-phycocyanin, 24 ps for allophycocyanin and 50 ps for chlorophyll *a*.

A kinetic analysis of these results indicates that energy transfer between the pigments is at least 99% efficient and is governed by an exp $-At^{1/2}$ transfer function. The apparent exponential behaviour of the fluorescence decay functions of the latter three pigments is shown to be a direct result of the energy transfer kinetics, as are the observed risetimes in the fluorescence emissions.

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Abbreviation: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyl urea.

## Introduction

The red alga, *Porphyridium cruentum*, is a unicellular member of the lower rhodophyceae. It possesses water-soluble accessory light harvesting pigments that are contained within structures known as phycobilisomes attached to the thylakoid membrane [1]. Phycobilisomes contain three main pigments, namely B-phycoerythrin, R-phycocyanin and allophycocyanin; the presence of B-phycoerythrin [2], and allophycocyanin B [3] or an aggregated form of allophycocyanin [4] is still uncertain (see Part II). Each of these pigments fluoresce in a well-defined spectral region, and the emissions may be wavelength-resolved without difficulty [5]. Chlorophyll *a* in *P cruentum* is contained within the thylakoid membrane, as are the carotenoids, the most important of which is $\beta$-carotene [6].

Early steady-state fluorescence studies of energy transfer in *P. cruentum* [7] indicated that the phycobilisomes preferentially serve Photosystem II and subsequently the energy transfer sequence has been proposed as:

B-phycoerythrin → R-phycocyanin → allophycocyanin → chlorophyll *a*

(an excellent review on the phycobilins can be found in ref. 5). Probably as a result of this highly efficient light harvesting system, Photosystem II has less chlorophyll *a* associated with it than Photosystem I [8]. The fluorescence lifetimes of chlorophyll *a* and the accessory pigments in *P. cruentum* were investigated some time ago by Rabinowitch and co-authors, who used a nanosecond flashlamp technique which entailed deconvolution of the fluorescence decay from the flashlamp profile [9,10,11]. B-phycoerythrin was found to transfer energy to R-phycocyanin with a transfer time of 300 ± 200 ps, and the transfer times of R-phycocyanin to chlorophyll *a* via allophycocyanin was reported to be 500 ± 200 ps [10]. Considering the efficiency of energy transfer [7] and the square root of time dependence of the transfer kinetics [12,13], it seems probable that the transfer times are much shorter than those reported. Similarly, the kinetic analysis presented [10] needs to be modified to include the non-exponential behaviour of the transfer kinetics. Previous studies of the chlorophyll *a* fluorescence kinetics have used excitation with blue light to reduce the interference from the overlapping phycobilin emission [14], and chlorophyll lifetimes close to 0.5 ns have been reported for the dark adapted state [11,15]. Mar et al. [16] have measured a chlorophyll fluorescence lifetime of approximately 1 ns for *P. cruentum* with the Photosystem II reaction centres fully closed, from which they deduced that the lifetime for the dark adapted state should be in the region of 350 ps.

In this communication we report our investigation of energy transfer between the accessory pigments and chlorophyll *a* in *P. cruentum* using picosecond time-resolved fluorescence spectroscopy.

## Materials and Methods

Cultures of *P. cruentum* were grown at room temperature (20—50° C) under continuous illumination from an incandescent lamp (1 mW/cm$^2$) supplemented

with daylight. The culture medium, a sterile artificial sea water medium [17], was agitated by bubbling with filtered air. Cells were normally harvested 10—12 days after innoculation by centrifugation at 3000 × *g* for 2 min. When young cells were required, the culture was innoculated with the minimum amount of a previous culture and the cells were harvested after 2—3 days growth when the cell density was still low. Gantt and Lipschultz [18] have shown that these young cells have a higher ratio of chlorophyll *a* : phycobilins in comparison with 5—12 day old cultures. The harvested cells were resuspended in growth medium to give a transmission of approximately 50% at 530 nm in a 1 mm cuvette. Measurements were performed with dark adapted *P. cruentum*, except in the case of chlorophyl *a* where the emission was also studied with the Photosystem II reaction centres fully closed by the addition of $10^{-4}$ M DCMU and continuous irradiation with 633 nm light (1.25 mW/cm$^2$) from a CW helium: neon laser. Young cells were used only when expressly stated.

The picosecond laser and streak camera system have been described in detail elsewhere [12,13,19]. A train of 6 ps (full width at half maximum height), 530 nm light pulses is generated by a frequency-doubled, mode-locked neodymium: glass laser oscillator. A single pulse is extracted from the centre of the train by a Pockels cell electro-optic shutter. The intensity of the 530 nm excitation pulse was controlled by a non-saturable neutral optical density filter situated before the sample. An area of 0.28 cm$^2$ of the sample was irradiated which limited the maximum unattenuated intensity to $10^{15}$ photons/cm$^2$; in general the intensity was kept below $10^{14}$ photons/cm$^2$ for these measurements. Fluorescence from the sample was passed through a wavelength selection filter and focussed onto the slit of an S20 photocathode Imacon 600 streak camera (John Hadland (P.I.) Ltd.). A vidicon optical multichannel analyser (OMA 1205 A and B, Princeton Applied Research) stored the resulting streak trace in digital form which could then be displayed on an oscilloscope or transferred to punch tape for analysis. Streak speeds were varied between 120 ps/50 OMA channels (120 ps per major division on the oscilloscope traces) and 540 ps/50 OMA channels depending upon the duration of the fluorescence decay. The linearity of the detection system is better than ±3% between 30 and 3000 counts in any channel of the OMA memory. Fluorescence emission components from *P. cruentum* were resolved with the following filters:

(i) B-phycoerythrin — 576 nm, 9 nm bandwidth Balzer B-40 Filtraflex interference filter.

(ii) R-phycocyanin — 640 nm, 13 nm bandwidth Balzer B-40 Filtraflex interference filter.

(iii) allophycocyanin — 661 nm, 14 nm bandwidth MTO Intervex A interference filter.

(iv) chlorophyll *a* — 685 nm, 11 nm bandwidth Balzer B-40 Filtraflex interference filter.

Fluorescence emission and excitation spectra were recorded on a Perkin-Elmer MPF-3 spectrofluorimeter, and were not corrected for the spectral response of the photomultiplier (Hamamatsu R446S) or the monochromator, or for the emission spectrum of the xenon arc lamp.

## Results

*Spectral characteristics*

The absorption spectra of the *P cruentum* cultures grown for these experiments (see Fig. 1) agreed with previously published spectra of this alga. Steady-state fluorescence emission and excitation spectra of the intact algal cells, under the conditions used in the kinetic measurements, are shown in Fig. 2. The 10—12 day old cultures gave a relatively high intensity B-phycoerythrin emission band at 578 nm (Fig. 2A) upon excitation at 530 nm. Only a small change was observed in the chlorophyll *a* emission at 685 nm when 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU) was added to these older cultures, as shown in Fig. 2A. In contrast, the younger cultures (2—3 days old) gave a much larger change in the emission intensity at 685 nm on DCMU addition (Fig. 2B). The emission intensities of B-phycoerythrin (578 nm), R-phycocyanin (640 nm) and allophycocyanin (660 nm) from the young cells also showed slight differences from those obtained from the older cells. It is apparent that excitation of B-phycoerythrin with 530 nm light results in energy transfer to the other pigments, as these do not have a significant absorption at this wavelength. The fluorescence from these pigments can be wavelength-resolved with suitable interference filters, although a certain amount of overlap is unavoidable. Fluorescence emission spectra of the isolated phycobiliproteins are well documented [2] and the reported fluorescence maxima are close to those observed in the spectra of these intact cells.

Excitation spectra of *P. cruentum* at various monitoring wavelengths are shown in Fig. 2C-F; at each wavelength the B-phycoerythrin band is predominant and the large amount of chlorophyll *a* observed in the absorption spectrum does not contribute significantly to the fluorescence emission in agreement with previous studies [7]. However, a weak chlorophyll *a* contribution

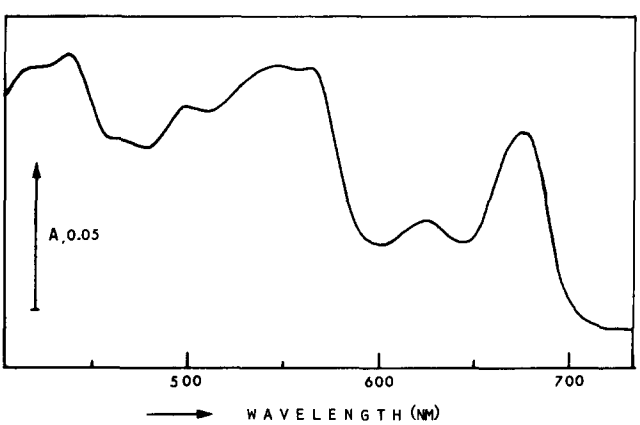


Fig. 1. The absorption spectrum of *Porphyridium cruentum*. A dilute suspension of 10—12 day old cells, pathlength 10 mm, was measured in an Aminco-Chance DW2 spectrophotometer with opal glass to reduce scattering artefacts. The peak at 678 nm is due to chlorophyll *a* only, that at 625 nm is due both to chlorophyll *a* and R-phycocyanin. B-phycoerythrin contributes the intense twin peak at 565—550 nm, and carotenoid absorption bands are apparent at 500 and 470 nm. The major band in the blue at 436 nm is the Soret band of chlorophyll *a*.

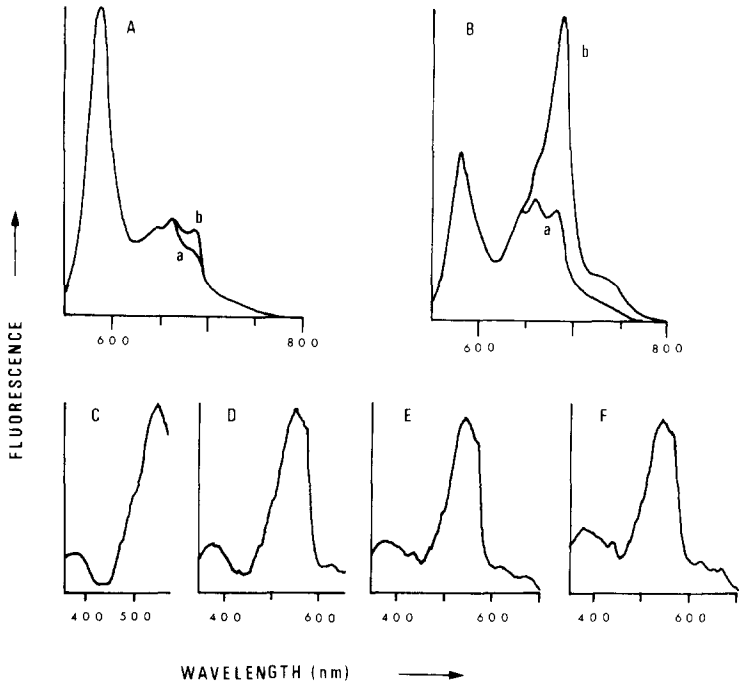


Fig. 2. The fluorescence emission and excitation spectra of *P. cruentum* measured on a Perkin Elmer MPF 3 fluorescence spectrophotometer using wavelengths employed in fluorescence kinetics experiments. In (A) and (B) the emission spectrum on excitation at 530 nm is shown for normal and young cultures respectively both without addition (a), and also on addition of $10^{-4}$ M DCMU (b). In (C)—(F) the excitation spectra for emission at 576 nm (C), 661 nm (D) and 730 nm (E and F) are shown. The excitation spectrum for 640 nm emission was similar to (D). Chlorophyll emission was monitored in young cells at 730 nm rather than 685 nm, both with out addition (E), and on addition of $10^{-4}$ M DCMU (F). No corrections were made for photomultiplier response, monochromator sensitivity or xenon arc lamp emission. Fluorescence intensity is expressed on a linear scale.

was observed for emission at 730 nm as indicated by the small peak at 436 nm which corresponds to the chlorophyll Soret band (Fig. 2E and 2F). When DCMU was added, the chlorophyll *a* and carotenoid contribution to the 730 nm fluorescence (light absorbed in the region of 350—450 nm) increased. Since these pigments are primarily associated with Photosystem I [20,21], this may indicate an increased contribution from Photosystem I to the emission at 730 nm. Minor peaks observed at 620 nm and 650 nm can be ascribed to R-phycocyanin and allophycocyanin respectively.

*Fluorescence kinetics*

Fluorescence decay curves for the three accessory pigments, B-phycoerythrin, R-phycocyanin and allophycocyanin, are shown in Fig. 3; all of these traces were obtained by single pulse excitation at 530 nm with an intensity of less than $10^{14}$ photons/cm$^2$. The fluorescence kinetics of B-phycoerythrin were found to follow an exp $-At^{1/2}$ decay law, similar to that reported for the fluorescence decay of chlorophyll *a* in *Chlorella* and spinach chloroplasts [12,13]. However, the emissions from R-phycocyanin and allophycocyanin both appeared to be governed by an exp $-kt$ decay law. The measured fluorescence

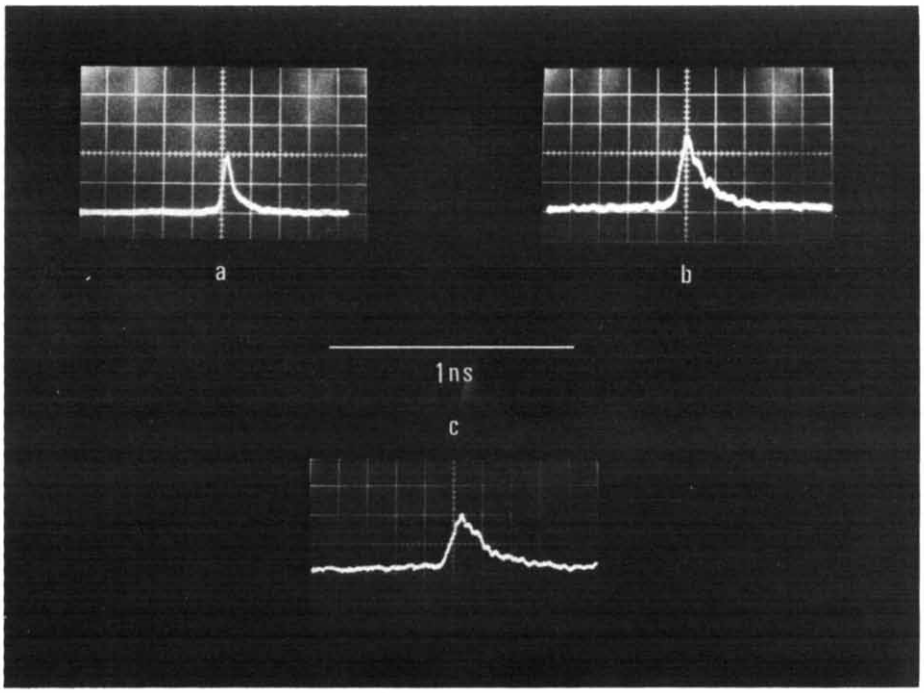


Fig. 3. The wavelength-resolved fluorescence kinetics of phycobiliproteins in *P. cruentum* measured on the picosecond laser apparatus. The monitoring wavelengths were a, 576 nm for B-phycoeryhthrin; b, 640 nm for R-phycocyanin; and c, 661 nm for allophycocyanin. An upward deflection represents an increase in fluorescence intensity on a linear scale. For further details see text.

lifetimes were 70 ps (mean lifetime) for B-phycoerythrin, 90 ps for R-phycocyanin and 118 ps for allophycocyanin. Since exciton-exciton annihilation could cause severe distortion of the decay traces [12,13], the measurements were repeated with a cut-off filter that transmitted above 600 nm (RG 600,

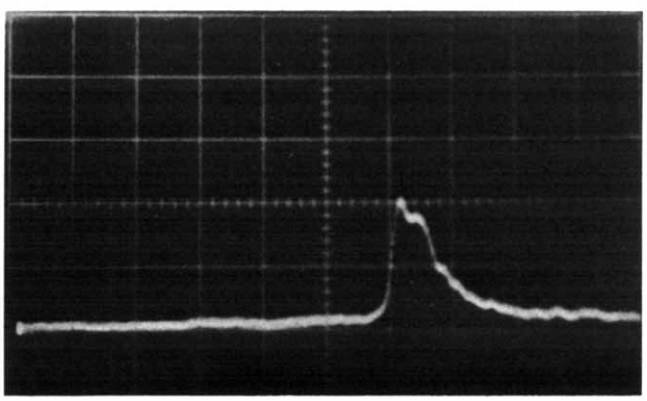

Fig. 4. The fluorescence kinetics of the photosynthetic pigments of *P. cruentum* at a low laser pulse intensity. A red cut-off wavelength selection filter was used (50%T at 600 nm), and the photon density was $10^{13}$ photon/cm$^2$. An upward deflection represents an increase in fluorescence intensity on a linear scale. Each major division on the x-axis represents 188 ps.

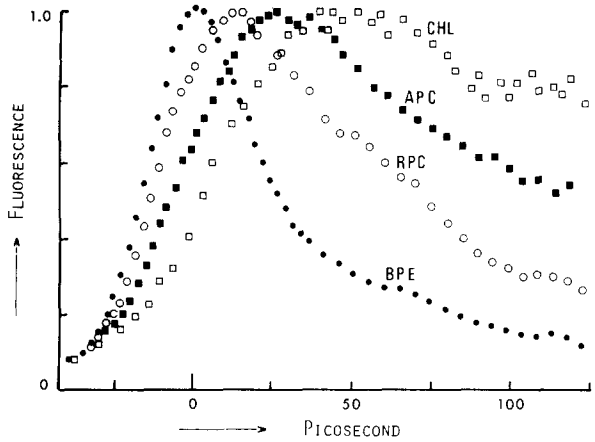


Fig. 5. The risetimes of wavelength-resolved pigment fluorescence in *P. cruentum*. Each curve was measured separately with interference filters as wavelength selection filters, and aligned on the time axis as described in the text. BPE is B-phycoerythrin, RPC is R-phycocyanin, APC is allophycocyanin and CHL is chlorophyll *a*. Fluorescence intensity is on a normalised linear scale.

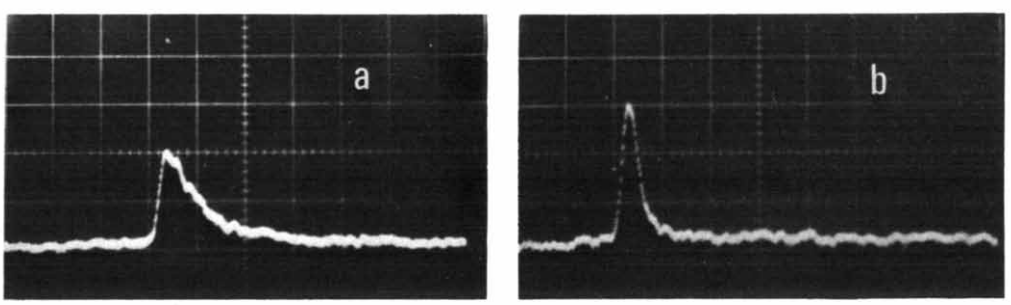


Fig. 6. The fluorescence kinetics of chlorophyll *a* in dark-adapted young cells of *P. cruentum*. In (a) on a short timescale (188 ps per major division), and on a longer timescale in (b) (543 ps per major division) to show the absence of a long component. A Balzer B40-685 nm interference filter was used as wavelength selection filter, and an upward deflection represents an increase in fluorescence.

Schott) which enabled the excitation intensity to be reduced to $10^{13}$ photons/$cm^2$ (see Fig. 4). Although the fluorescence components were not wavelength-resolved, there was no indication that the decay rates were slower than those observed at $10^{14}$ photons/$cm^2$. The reproducible decay curve shown in Fig. 4 indicated that at least one of the fluorescence components had a significant risetime. The initial part of the wavelength-resolved decay of each of the pigments is shown in Fig. 5; the risetime of the B-phycoerythrin emission was consistent with the time resolution of the detection system and the width of the excitation pulse, whereas those of R-phycocyanin and allophycocyanin were significantly longer. After matching the initial rise of the fluorescence emissions with that of B-phycoerythrin, the times taken to reach maximum emission intensity, relative to the maximum of B-phycoerythrin, were approximately 12 ps and 24 ps for R-phycocyanin and allophycocyanin respectively.

The fluorescence emission from chlorophyll *a* in the 10—12 day old cultures of *P. cruentum* was relatively weak and was obscurred by the strong allophycocyanin emission. Consequently, young cells (2—3 days old) were used to study the kinetics of the chlorophyll *a* emission. The chlorophyll fluorescence decay traces obtained from these cultures are shown in Figs. 6 and 7; Fig. 6 shows the decay from the dark adapted alga and Fig. 7 the decay after treatment with DCMU and preillumination. The fluorescence decay kinetics of the 685 nm component (predominantly chlorophyll *a*) appeared to be adequately described by an exp $-kt$ decay law with a lifetime of 175 ps. A risetime to maximum emission of 50 ps was measured for chlorophyll *a* and is shown with the other pigments in Fig. 5. Upon the addition of DCMU and preillumination the decay curves became nonexponential as shown in Fig. 7. The initial phase of this decay had a 1/e lifetime of 110 ps, and the longer component had a lifetime of 840 ps.

A summary of the observed fluorescence decay rates lifetimes and risetimes is given in Table I.

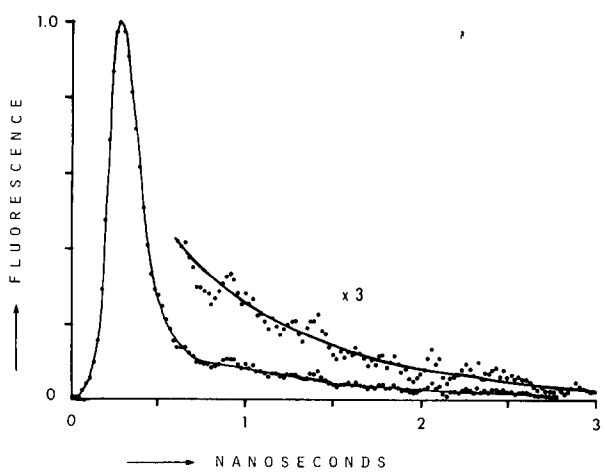


Fig. 7. The fluorescence kinetics of chlorophyll *a* in young cells of *P. cruentum* with Photosystem II reaction centres closed by addition of DCMU and preilluminated as described in the text. Other conditions as for Fig. 6.

TABLE I

THE FLUORESECENCE CHARACTERISTICS OF THE PIGMENTS OF *PORPHYRIDIUM CRUENTUM*

a, mean lifetime; b, calculated rate constants for fluorescence decay by direct excitation; c, apparent rate constants caused by energy transfer; *, calculated values obtained from the kinetic equations using the $A$ values (see text); (i), dark adapted algae; (ii), algae treated with DCMU and preillumination; $A$ and $k$ are fluorescence decay constants; $\tau_{1/e}$ is the 1/e fluorescence lifetime; $\phi_{calc}$ is the quantum of fluorescence calculated from the measured fluorescence decay constants; $\tau_{rise}$ is the risetime of fluorescence.

| Pigment | Emission wavelength (nm) | $\tau_{1/2}$ (ps) | $A$ ($ps^{-1/2}$) | $k$ ($ps^{-1}$) | $\tau_{rise}$ (ps) | $\tau^*_{1/e}$ (ps) | $\tau^*_{rise}$ (ps) | $\phi_{calc}$ |
|---|---|---|---|---|---|---|---|---|
| B-phycoerythrin | 578 | 70 ± 5 [a] | 0.26 | — | 0 | — | 0 | 0.0036 |
| R-phycocyanin | 640 | 90 ± 10 | 0.48 [b] | 0.0110 [c] | 12 | 85 ± 5 | 12 | 0.0029 |
| Allophycocyanin | 660 | 118 ± 8 | 0.52 [b] | 0.0085 [c] | 24 | 115 ± 8 | 22 | 0.0018 |
| Chlorophyll *a* (i) | 685 | 175 ± 10 | 0.40 [b] | 0.0057 [c] | 50 | 176 ± 8 | 52 | 0.0007 |
| Chlorophyll *a* (ii) | 685 | 110 ± 5 | — | 0.0091 | — | — | — | |
| | 685 | 840 ± 10 | — | 0.0012 | — | — | — | 0.0021 |

## Discussion

We have previously shown that energy transfer in the chlorophyll *a* antenna system of Photosystem II results in a fluorescence decay law of the form [12, 13,22];

$$I(t) = I_0 \exp - 2At^{1/2} \tag{1}$$

where $I_0$ is the initial fluorescence intensity and $I(t)$ is the fluorescence intensity at time $t$. The rate constant, $2A$, is a combination of the rate constant controlling donor:donor energy transfer and that controlling donor:acceptor energy transfer. Since B-phycoerythrin also has a fluorescence decay law of this form, it may not be unreasonable to assume that energy transfer among the other light harvesting pigments of *P. cruentum* is kinetically similar. As yet it is not possible to draw any conclusions concerning the validity of the Förster equation for energy transfer [23] in these systems. However, the general technique described by Birks [24] may be used to extend the kinetics implied by Eqn. 1 to a multiple donor:acceptor system.

If we consider a four component energy transfer system, where the pigments J, K, L and M transfer their energy in the following sequence:

$$J \rightarrow K \rightarrow L \rightarrow M \rightarrow X$$

where X is a non-fluorescent quencher such as the reaction centre, then direct excitation of any pigment will produce fluorescence emission with a decay law given by Eqn. 1; it is assumed that fluorescence emission and intersystem crossing only make a very minor contribution to the depletion of the excited state population. For direct excitation the rate constants for the decay of the excited state population of J, K, L and M are $A_1$, $A_2$, $A_3$ and $A_4$ respectively. Excitation of pigment J with a light pulse described by the function $P(t)$ will produce an excited state population $J^*$ whose decay is described by the rate equation:

$$\frac{dJ^*}{dt} = P(t) - J^* \cdot A_1 \cdot t^{-1/2}$$

If $P(t)$ is a $\delta$-pulse, then this function may be neglected for $t > 0$. Since the energy lost by J is gained by pigment K, the rate equation for the excited state population of K (i.e. $K^*$) is:

$$\frac{dK^*}{dt} = J^* \cdot A_1 \cdot t^{-1/2} - K^* \cdot A_2 \cdot t^{-1/2}$$

Substituting for $J^*$ using eqn. 1, the equation becomes:

$$\frac{dK^*}{dt} + K^* \cdot A_2 \cdot t^{-1/2} = J_0^* \cdot A_1 \cdot t^{-1/2} \cdot \exp - 2A_1 t^{1/2}$$

where $J_0^*$ is the excited state population of J at time $t = 0$. Solving the differential equation the time course of $K^*$ is given by:

$$K^*(t) = J_0^* \cdot A_1 \cdot \exp(2A_2 t^{1/2}) \cdot \int_0^t t^{-1/2} \cdot \exp(2A_2 - 2A_1) t^{1/2} \, dt$$

which gives

$$K^*(t) = \frac{J_0^* \cdot A_1}{(A_2 - A_1)} [\exp(-2A_1 t^{1/2}) - \exp(-2A_2 t^{1/2})] \tag{2}$$

Similarly the rate equation for the excited state population of L is:

$$\frac{dL^*}{dt} = K^* \cdot A_2 \cdot t^{-1/2} - L^* \cdot A_3 \cdot t^{-1/2}$$

which, after substitution for $K^*$ from eqn. 2 becomes:

$$\frac{dL^*}{dt} + L^* \cdot A_3 \cdot t^{-1/2} = \frac{J_0^* \cdot A_1 \cdot A_2}{(A_2 - A_1)} \cdot t^{-1/2} \, [\exp(-2A_1 t^{1/2}) - \exp(-2A_2 t^{1/2})]$$

Again solving the differential equation the function $L^*(t)$ has the form:

$$L^*(t) = \frac{J_0^* \cdot A_1 \cdot A_2}{(A_2 - A_1)} \left[ \frac{\exp(-2A_1 t^{1/2}) - \exp(-2A_3 t^{1/2})}{(A_3 - A_1)} \right.$$

$$\left. - \frac{\exp(-2A_2 t^{1/2}) + \exp(-2A_3 t^{1/2})}{(A_3 - A_2)} \right] \tag{3}$$

and by the same method the function $M^*(t)$ is:

$$M^*(t) = \frac{J_0^* \cdot A_1 \cdot A_2 \cdot A_3}{(A_2 - A_1)} \left[ \frac{\exp(-2A_1 t^{1/2}) - \exp(-2A_4 t^{1/2})}{(A_4 - A_1)(A_3 - A_1)} \right.$$

$$- \frac{\exp(-2A_4 t^{1/2}) + \exp(-2A_3 t^{1/2})}{(A_3 - A_4)(A_3 - A_1)}$$

$$\left. - \frac{\exp(-2A_4 t^{1/2}) + \exp(-2A_2 t^{1/2})}{(A_2 - A_4)(A_3 - A_2)} + \frac{\exp(-2A_4 t^{1/2}) - \exp(-2A_3 t^{1/2})}{(A_3 - A_4)(A_3 - A_2)} \right] \tag{4}$$

The final form of the Eqns. 2—4 are similar to those derived by Tomita and Rabinowitch [10], except that these are all dependent upon the square root of time. It should be noted that these equations are probably not valid for long time intervals after excitation since the normal fluorescence decay of the pigment will eventually become significant. In fact, the streak camera's sensitivity to incident light precludes measurements in this region and the approximation should describe the observable initial fluorescence decay. Since $A_1$ is given by the fluorescence decay rate of B-phycoerythrin, the values of $A_2$, $A_3$ and $A_4$ may be evaluated sequentially so that each gives the experimentally recorded fluorescence decay rate and risetime when inserted into the relevant equation. The rate constants that gave the best fit to the experimental data were as follows, $2A_1 = 0.26\ ps^{-1/2}$, $2A_2 = 0.48\ ps^{-1/2}$, $2A_3 = 0.52\ ps^{-1/2}$ and $2A_4 = 0.40\ ps^{-1/2}$. Apart from Eqn. 1, all of the equations gave decay curves which were very close to a normal exponential decay of the form $\exp -kt$; the slight deviation from exponentiality would not have been discernable on the experimental decay curves. In order to demonstrate the agreement between the calculated and experimental data, the calculated curves were convoluted with the resolution function of the streak camera. This function is a Gaussian curve with a full width, at half maximum height, of 28 ps on this time scale and was determined from the apparent profile of a 6 ps laser pulse recorded at the same streak speed. Fig. 8 shows the convoluted theoretical decay curves of the four pigments obtained from the numerical evaluation of the expression:

$$F(t) = \int_0^\infty f(\alpha) \cdot R(t - \alpha)\mathrm{d}\alpha$$

where $F(t)$ is the convoluted fluorescence function, $f(\alpha)$ is the true fluorescence function, $R(t)$ is the camera resolution function and $\alpha$ is the time spread

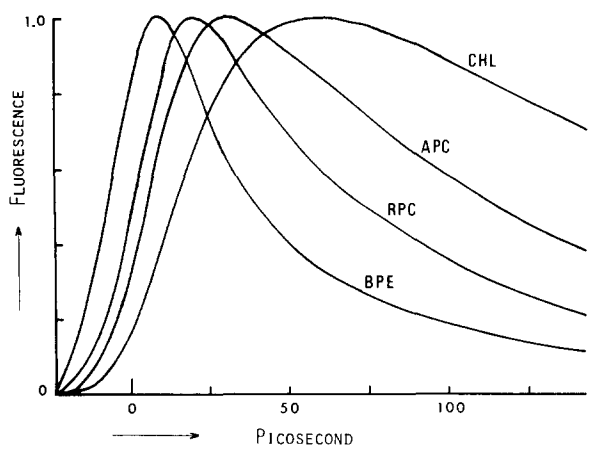


**Fig. 8. Theoretical curves for the rise and decay of fluorescence of the pigments of *P. cruentum* on direct excitation of B-phycoerythrin and transfer of excitation to the other pigments according to the kinetic treatment derived in the text. Other conditions as for Fig. 5.**

of the resolution function. This procedure only affects the fluorescence function of B-phycoerythrin to any significant extent, but does distort the initial rise in the fluorescence of the remaining curves. However, the time intervals between the maxima of the curves were not altered significantly. A summary of the calculated rate constants, apparent fluorescence lifetimes and risetimes is given in Table I for comparison. Within the limits of experimental error the calculated curves are in reasonable agreement with those observed experimentally.

The fluorescence quantum yield of each of the pigments when subjected to direct excitation, may therefore be calculated from the expression:

$$\phi_{calc} = \frac{\phi_0}{\tau_0} \int_0^\infty \exp(-2At^{1/2})dt = \frac{2\phi_0}{\tau_0 \cdot (2A)^2}$$

where $\phi_0$ and $\tau_0$ are the fluorescence quantum yield and lifetime respectively of the pigment in vitro. Brody and Rabinowitch [9] have reported the fluorescence lifetimes of B-phycoerythrin and R-phycocyanin in vitro to be 7.1 ns ($\phi_0$ = 0.85) and 1.8 ns ($\phi_0$ = 0.53) respectively; the lifetime of chlorophyll *a* is 5.7 ns ($\phi_0$ = 0.33) [12]; the radiative lifetime of allophycocyanin was estimated to be 4.2 ns from the results obtained in Part II of this work. The values of $\phi_{calc}$ obtained from these results and the calculated decay rates for each pigment are summarised in Table I. Since the calculated fluorescence quantum yields are all less than 0.01, the assumption that fluorescence emission only makes a minor contribution to the depletion of the excited state population appears to be valid over a major part of the excited state decay profile. The calculated quantum yields also indicate that energy transfer between any two pigments in the sequence is approximately 99% efficient, in contrast to the transfer efficiencies reported by Tomita and Rabinowitch [10], (phycoerythrin:phycocyanin 96 ± 3%, phycocyanin:allophycocyanin:chlorophyll *a* 78 ± 8%).

The pigment b-phycoerythrin [2] has been omitted from the discussion since its spectral characteristics are so similar to those of B-phycoerythrin that the two pigments may be considered to operate in parallel. Hence the rate constant $A_1$ includes the rate constants for both pigments acting in parallel. The existence of allophycocyanin B [3] or an aggregated form of allophycocyanin [4] in intact *P. cruentum* remains to be confirmed. However, this pigment would probably have a very high transfer efficiency to chlorophyll *a*, and would not affect the observed kinetics to any great extent. Reabsorption of the fluorescence emission by the pigments should not result in any distortion of the fluorescence decay kinetics since excitation by this process can only produce less than 1% of the total excited state population. Similarly, direct energy transfer from B-phycoerythrin to allophycocyanin or chlorophyll *a* and from R-phycocyanin to chlorophyll *a* are minor processes owing to the very small overlap between the emission and absorption spectra of these pigments [2].

The absence of exciton-exciton annihilation at the intensities used in these measurements is probably a direct result of the transfer kinetics and the geometry of the phycobilisome. Gantt et al. [4] suggested that the allophycocyanin is in contact with the thylakoid membrane and surrounded by a layer of R-phycocyanin, which in turn is surrounded by a layer of B-phycoerythrin. In this structured sphere energy migration between layers is more probable than migra-

tion within the layer. For an initial random distribution of excitons in the B-phycoerythrin layer, the probability of exciton-exciton interactions is much lower than for a comparable situation in a chlorophyll *a*-containing antenna system. The relative magnitudes of the rate constants for energy transfer also preclude the formation of a large exciton population in R-phycocyanin, allophycocyanin and chlorophyll *a* in the dark-adapted state. Clearly exciton-exciton annihilation will only be observed at very high excitation intensities ($>10^{15}$ photons/cm$^2$) or when energy transfer between the pigments is prevented, as in the case of isolated phycobilisomes described in part II.

The energy transfer rate constant found for the chlorophyll *a* of Photosystem II in the dark adapted state, 0.40 ps$^{-1/2}$, is much larger than the value of 0.047 ps$^{-1/2}$ observed in both *Chlorella* [12] and spinach subchloroplast fragments [13]. This could be explained by the smaller amount of chlorophyll associated with Photosystem II in *P. cruentum*, which would reduce the migration distance to an active reaction centre and consequently increase the trapping rate. Ley and Butler [8] have reported that the quantum yield of energy transfer from Photosystem II to I is high in dark-adapted *P. cruentum*, which might also cause a faster Photosystem II fluorescence decay compared to that in *Chlorella*.

Closure of the Photosystem II reaction centres with DCMU and preillumination resulted in complex decay kinetics for chlorophyll fluorescence (Fig. 7). It is possible that the shorter component is due to energy transfer from Photosystem II to I, for it has been reported that the quantum yield of this process increases to 0.90—0.95 with the Photosystem II reaction centres closed [8]. Thus the initial component ($k$ = 0.0091 ps$^{-1}$, $\tau_{1/e}$ = 110 ps) might reflect this Photosystem II to I energy transfer, whereas the second component ($k$ = 0.0012 ps$^{-1}$, $\tau_{1/e}$ = 840 ps) would represent the chlorophyll fluorescence lifetime of Photosystem II with reaction centres closed. Alternatively it is possible that exciton-exciton annihilation might become more important under these conditions owing to the small size of the Photosystem II chlorophyll antenna system in *P. cruentum* and the longer lifetime of excitons on addition of DCMU and preillumination. However, when the laser pulse intensity was varied, although the relative intensities of the two components changed slightly the decay rate of the initial component altered only slightly and not to the appreciable extent expected from previous observations of exciton-exciton annihilation [12,13,22]. Therefore the contribution of exciton-exciton annihilation to the observed decay law would not appear to be a major factor at these excitation intensities ($10^{14}$ photons/cm$^2$).

A study of the kinetics of energy transfer within the isolated phycobilisome is reported in the accompanying paper (part II).

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