PICOSECOND ENERGY TRANSFER IN *PORPHYRIDIUM CRUENTUM* AND *ANACYSTIS NIDULANS*

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ABSTRACT Picosecond energy transfer is measured in *Anacystis nidulans* and *Porphyridium cruentum*. Fluorescence is sensitized by a 6-ps laser flash, at 530 nm. The time dependence of fluorescence is measured with reference to the laser pulse. Fluorescence is recorded from phycoerythrin (576 nm), R-phycocyanin (640 nm), allophycocyanin (666 nm), Photosystem II chlorophyll (690 nm) and long wave length chlorophyll (715 nm). Energy transfer measurements are made at 37°C, 23°C, 0°C, and 77°K. It is shown that the rate of energy transfer can be varied with temperature. In both *A. nidulans* and *P. cruentum* there is a sequential transfer of excitation energy from phycoerythrin to phycocyanin to allophycocyan to Photosystem II chlorophyll fluorescence. The long wavelength chlorophyll fluorescence at 715 nm, however, does not always follow a sequential transfer of excitation energy. Depending on the temperature, fluorescence at 715 nm can precede fluorescence from phycocyanin.

INTRODUCTION

Notable strides have been made in photosynthesis using picosecond techniques (Holton and Windsor, 1978). The primary picosecond processes involving energy transfer appear to have a crucial influence on the overall efficiency of photosynthesis. The assortment of phycobilin and chlorophyll pigments in the red and blue green algae make them ideal organisms in which to study the mechanism of energy transfer and the distribution of excitation energy between Photosystem I and Photosystem II.

The first significant quantitative study of energy transfer in red and blue-green algae was carried out by Duysens (1951) using fluorescence excitation spectra. He showed that in *Porphyridium cruentum* the efficiency of energy transfer from the phycobiliproteins to chlorophyll was 90% or better. The transfer efficiency and photosynthetic action spectrum in *P. cruentum* is not a static or constant property but can be modified (e.g., by pretreatment of the organism to various colors and intensity of light [Brody and Brody, 1959, 1962]).

The rate of energy transfer from phycobiliproteins to chlorophyll *in situ*, which is in the picosecond range, can be directly measured. The first reported measurement of the rate of intramolecular energy transfer gave an upper limit of 500 ps, as the time for energy absorbed by phycoerythin to be emitted as fluorescence by chlorophyll (Brody, 1960). Tomita and Rabinowitch (1962) reported that the time required to transfer energy from phycoerythrin to phycocyanin was 300 ps and that the time required to transfer energy from phycocyanin to chlorophyll *a* was 500 ps. In *Anacystis nidulans* they reported that the time required to transfer energy from phycocyanin to chlorophyll *a* was 500 ps.

BIOPHYS. J. © Biophysical Society · 0006-3495/81/06/439/11 $1.00
Volume 34 June 1981 439–449

Birks (1968) derived equations describing the transfer of energy between fluorescent molecules in a ternary system. Porter et al., (1978) extended the technique used by Birks (1968) to describe the kinetics of energy transfer from donor to acceptor. The final equations obtained by Porter et al. (1978) describing the fluorescence kinetics of the various pigments in *P. cruentum* were similar to those obtained by Tomita and Rabinowitch (1962). Using a picosecond streak camera Porter et al. (1978) resolved the time required to sequentially transfer energy absorbed by B-phycoerythin to R-phycocyanin, then to allophycocyanin, and finally to chlorophyll. They found that the rise time to the maximum of fluorescence for R-phycocyanin (640 nm) was 12 ps, for allophycocyanin (660 nm) 24 ps, and for chlorophyll (685 nm) 50 ps. They concluded that the sequential change in rise times of the fluorescence emissions was a result of the energy transfer kinetics.

The phycobilin pigments appear to be contained in a structure known as a phycobilisome. The structure of the phycobilisome was proposed by Gantt (1977), Gantt et al. (1976), and Bryant et al. (1979). An allophycocyanin core was surrounded by a hemispherical layer of phycocyanin which in turn was surrounded by a hemispherical layer of phycoerythrin.

MATERIALS AND METHODS

The instrumentation used in the present work to resolve picosecond fluorescence was described in detail by Tredwell et al. (1978) and Porter et al. (1978). A train of pulses was generated by a mode-locked Nd^{3+} glass laser. A frequency doubler gave 530-nm pulses. An electro-optic shutter selected a single, 6-ps pulse. Fluorescence from the sample passed through a filter into an Imacon 600 streak camera (John Hadland P.I. Ltd.; England). The fluorescence streak trace was detected by a 500-channel optical multichannel analyzer (OMA 1205 A and B, Princeton Applied Research, N. J.) and stored in its digital memory. The streak rate was 1.72 ps/channel. Data in the digital memory were transferred directly into a Data General computer (Data General Corp., Westboro, Mass.) where data reduction was carried out (summation of curves and graphing of results). Excitation intensity was $<2 \times 10^{14}$ photons cm^{-2} to avoid annihilation effects which shorten fluorescence life times and lower fluorescence yields (Porter et al., 1977).

The response of the streak camera to incident light was shown to be linear to better than ±3%; the streak rate across the region detected by the OMA was also shown to be constant (Treadwell, 1978).

Energy transfer in the algae *A. nidulans* and *P. cruentum* were measured relative to the laser pulse used to sensitize fluorescence. This procedure eliminated the problem of jitter in triggering the streak camera. Using a beam splitter, part of the laser pulse went directly to the streak camera and the remainder was used to irradiate the sample. The narrow laser pulse was used as a timing mark to measure the time for energy transfer. For each experiment nine fluorescence decays were summed together. The peaks of the laser pulses were made to coincide before summing the fluorescence curves.

Fluorescence components from *A. nidulans* and *P. cruentum* were resolved with the following filters: for B-phycoerythrin a 576-nm, 9-nm bandwidth Balzer interference filter; for R-phycocyanin a 640-nm, 13-nm bandwidth Balzer interference filter; for allophycocyanin a 666-nm, 14-nm bandwidth MTO interference filter; for Photosystem II chlorophyll a 690-nm, 11-nm bandwidth Balzer interference filter; for long wavelength chlorophyll (715 nm) an RG715 filter from Schott Optical Glass Inc., Duryea, Pa. The optical delay introduced by these filters is 24, 20, 23, 25, and 15 ps, respectively. The optical delay was readily calculated from the thickness and index of refraction of the filters. The data shown in this paper have been corrected for the delay introduced by the various filters. Fluorescence yields were <0.02.

To compare the fluorescence data from different experiments it was necessary to replot the data on graphs having the same dimensions. The magnitudes of the replotted fluorescence data were normalized. The effect of temperature on energy transfer was qualitatively compared by measuring the time at which the fluorescence reached its maximum. Since this was only a qualitative comparison the complete fluorescence curves are shown in this paper. For clarity, only every fourth datum was used in drawing the figures. To analyze the energy transfer curves requires various assumptions regarding the mechanism of energy transfer and the molecular arrangement of phycobilin and chlorophyll pigments, which are not within the scope of this paper.

The culture conditions used for the red and blue-green algae were described by Gantt and Lipschultz (1972) and Porter et al. (1978). Whole cells were used for all experiments. Three different batches of cells, grown under similar conditions, gave very similar results, i.e., for each wavelength the fluorescence peaks were within 3 ps of each other. All measurements were made in the absence of DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea] unless otherwise stated.

RESULTS

P. cruentum

At 23°C the time dependent sequence for transferring energy between the pigments is similar to that reported by Porter et al. (1978). The rise times and fluorescence maxima follow the sequence B-phycoerythrin, to R-phycocyanin, to allophycocyanin and then to chlorophyll (690 nm). The fluorescence maxima for B-phycoerythrin and chlorophyll (690 nm) are separated in time by 19 ps. The time dependence of fluorescence at 576 and at 690 nm, from *P. cruentum* at 23°C, is shown in Fig. 1.

The time dependence of fluorescence at 576, 666, 690, and 715 nm, at 37°C is shown in Fig. 2. The fluorescence maxima at 666 and 690 nm occur 13 and 53 ps, respectively, after the fluorescence maximum of B-phycoerythrin at 576 nm. Of particular interest is the observation that the fluorescence maximum at 715 nm precedes, in time, the fluorescence from

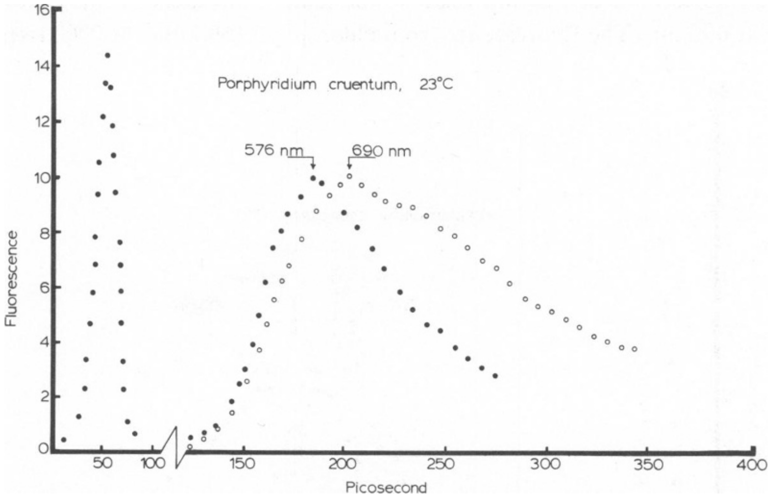

FIGURE 1 The time-dependent emission at 576 and 690 nm from *P. cruentum*, at 23°C. Fluorescence is excited with a single, 6-ps pulse at 530 nm. The intensity of the pulse is $<2 \times 10^{14}$ photons cm^{-2}. Using a beam splitter, part of the laser pulse goes directly to the streak camera (the narrow, far left, pulse shown above), the other part is used to irradiate the sample. Interference and colored filters are used to isolate fluorescence from the various pigments. For clarity, only every fourth datum is depicted in these figures. Fluorescence from B-phycoerythrin is detected at 576 nm, fluorescence from R-phycocyanin at 640 nm, from allophycocyanin at 666 nm, from Photosystem II chlorophyll at 690 nm, and from a long wavelength chlorophyll at 715 nm. To facilitate comparison of the fluorescence from the different pigments all curves are normalized to the same arbitrary maximum.

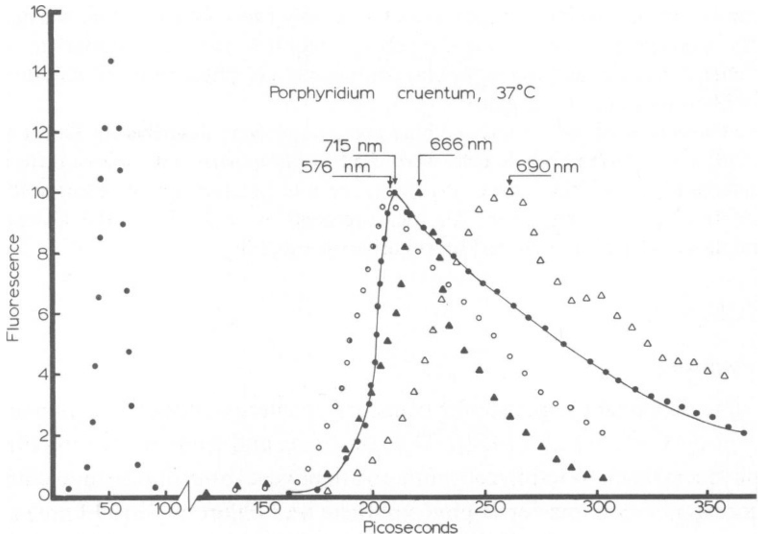

FIGURE 2 The time dependent emission at 576, 666, 690, and 715 nm from *P. cruentum* at 37°C. Other conditions are as described in Fig. 1.

allophycocyanin and chlorophyll (690 nm). The kinetics of fluorescence at 715 nm, which is quite reproducible, is not readily interpreted in terms of simple sequential transfer of energy.

The time dependence of fluorescence from *P. cruentum* at 0°C is shown in Fig. 3. It can be seen that the fluorescence at 715 nm rises slower than, and reaches a maximum after, the fluorescence at 690 nm. The fluorescence from chlorophyll (690 nm) at 0°C, rises faster and

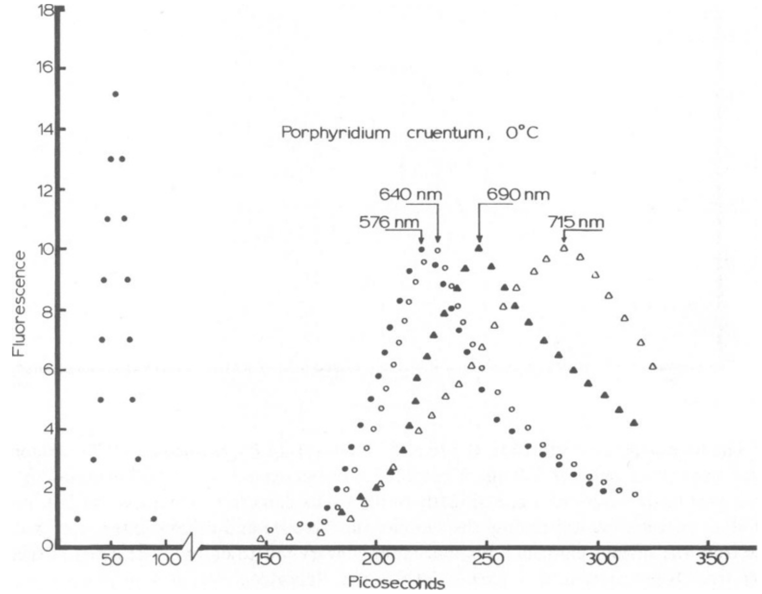

FIGURE 3 The time dependent emission at 576, 640, 690, and 715 nm from *P. cruentum*, at 0°C. Other conditions are as described in Fig. 1.

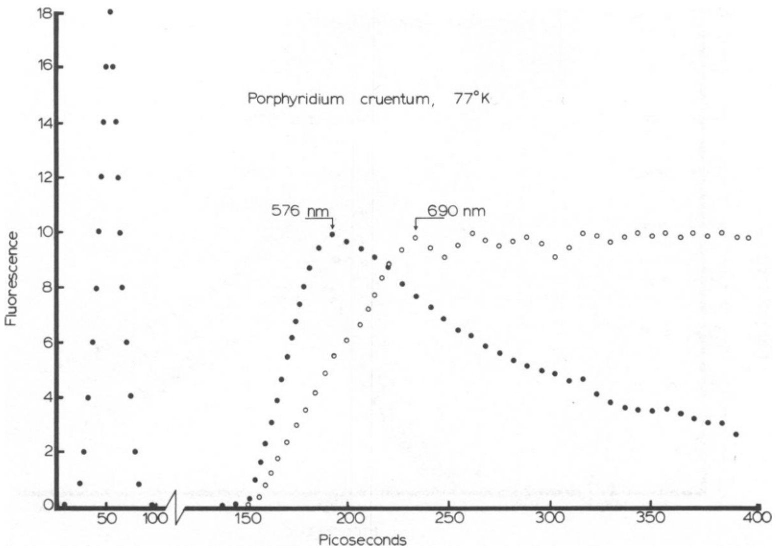

FIGURE 4 The time dependent emission at 576 and 690 nm from *P. cruentum* at 77°K. Other conditions are as described in Fig. 1.

reaches a maximum sooner than at 37°C. Fluorescence from R-phycocyanin (not shown) and allophycocyanin (666 nm) decays rapidly. The addition of 10^{-4} M DCMU to *P. cruentum* increases the time between the fluorescence maxima at 576 and 690 nm, from 26 to 40 ps.

Cooling *P. cruentum* to 77°K increases both the time required for energy transfer and the fluorescence life times (Fig. 4). The time between the maxima at 576 and 690 nm is 40 ps.

Data for *P. cruentum* are summarized in Table I.

A. nidulans

In the case of *A. nidulans* at 37°C there is a progressive shift of the fluorescence maxima and lengthening of the rise times for the fluorescence at 640, 666, and 690 nm (Fig. 5). The differences in time between the maximum at 640 nm and the maxima at 666 and 690 nm are

TABLE I
ENERGY TRANSFER TIMES IN *PORPHYRIDIUM CRUENTUM*

Temp	DCMU	Difference in time between the maximum at 576 nm and the flourescence maxima at:			
		640 nm	666 nm	690 nm	715 nm
		(ps)	*(ps)*	*(ps)*	*(ps)*
77°K	no	—	—	40	—
0°C	no	10	26	64	—
0°C	yes	—	—	40	—
23°C	no	—	—	19	—
37°C	no	—	13	53	2

The confidence limits for all time measurements are ±2 ps.

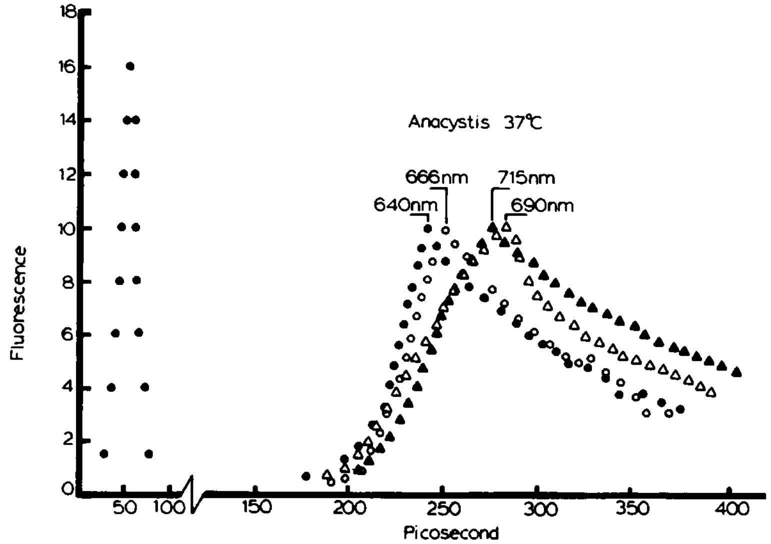

FIGURE 5 The time dependent emission at 640, 666, 690, and 715 nm from *A. nidulans* at 37°C. Other conditions are as described in Fig. 1.

8 and 40 ps, respectively. The difference in time between the maximum at 640 and at 715 nm is 34 ps; so that, the fluorescence at 715 nm preceeds the fluorescence at 690 nm.

Upon addition of 10^{-4} M DCMU, the difference in time between the fluorescence maximum at 640 nm and the maxima at 666, 690, and 715 nm is changed to 12, 16, and 20 ps, respectively, so that, in the presence of DCMU at 37°C, a normal sequence of energy transfer seems to obtain.

At 0°C the fluorescence maxima at 666, 690, and 715 nm occur at 4, 10, and 6 ps, respectively, after the maximum at 640 nm. The fluorescence at 715 nm preceeds the fluorescence at 690 nm. In the presence of 10^{-4} M DCMU, the rise time and maxima of fluorescence at 640 and 666 nm become essentially identical (Fig. 6). The decays, however, are different; it is faster for the 640-nm fluorescence and slower for 666-nm fluorescence. In the presence of 10^{-4} M DCMU, the maxima of fluorescence at 690 and at 715 nm are increased to 24 and 13 ps, respectively, after the maximum at 640 nm. In summary, the addition of DCMU (at 0°C) increases the time required to transfer energy to chlorophyll (690 nm). On the other hand, at 37°C, DCMU decreases the time required to transfer energy to chlorophyll (690 nm).

At 77°K there is an increase in the time required for energy transfer and the fluorescence life times (Fig. 7). The differences in time between the fluorescence maximum at 640 nm and the maxima at 666, 690, and 715 nm are 28, 65, and 75 ps, respectively. It was expected that the onset of fluorescence would be the same at all wavelengths, as seen under certain conditions (Figs. 1, 4, and 5). Nevertheless, the results showing a delay in onset of fluorescence in Figs. 2, 3, 6, and 7 are supported by the consistancy and reproducibility of the data.

Data for *A. nidulans* are summarized in Table II.

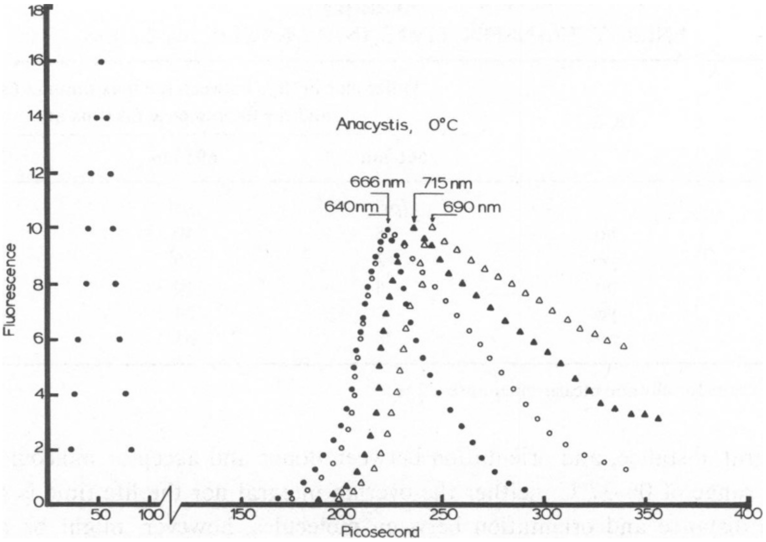

FIGURE 6 The time dependent emission at 640, 666, 690, and 715 nm from *A. nidulans* at 0°C. Other conditions are as described in Fig. 1.

DISCUSSION

In this work it is observed that the time for energy transfer between pigment molecules in *P. cruentum* and *A. nidulans* is not an invarient value. Rather, the transfer time can be varied by temperature or the addition of DCMU.

The rate of energy transfer between molecules is a function of fluorescence life time, the

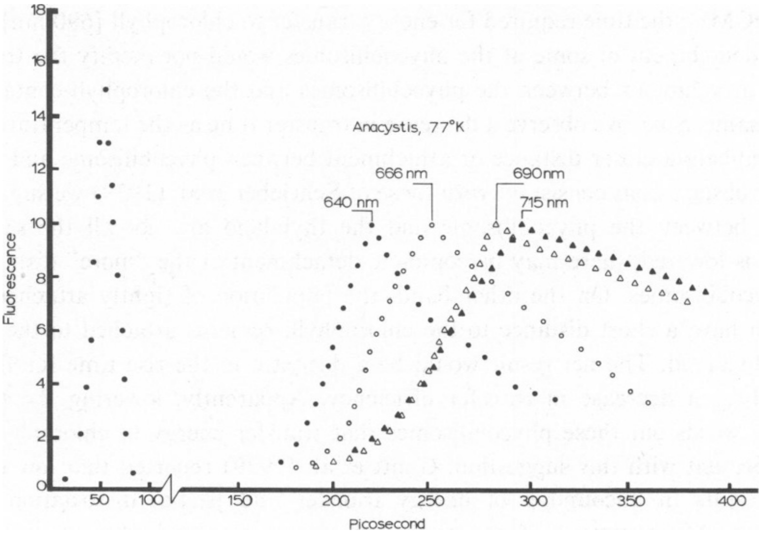

FIGURE 7 The time dependent emission at 640, 666, 690 and 715 nm from *A. nidulans* at 77°K. Other conditions are as described in Fig. 1.

TABLE II
ENERGY TRANSFER TIMES IN *ANACYSTIS NIDULANS*

Temp	DCMU	Difference in time between the maximum at 640 nm and the fluorescence maxima at:		
		666 nm	695 nm	715 nm
		(ps)	*(ps)*	*(ps)*
37°C	no	8	40	34
37°C	yes	12	16	20
0°C	no	4	10	6
0°C	yes	−2	24	13
77°K	no	28	65	75

The confidence limits for all time measurement are ±2 ps.

overlap integral, distance, and orientation between donor and acceptor molecules. Over the temperature range of 0°–37°C, neither the overlap integral nor the life time is significantly altered. The distance and orientation between molecules, however, might be modified by temperature.

From a study of the fluorescence spectra of *A. nidulans*, Schreiber et al. (1979) reported that a decrease in temperature results in a decrease in transfer efficiency between phycobilisomes and chlorophyll. They attributed the decrease in efficiency to a reversible, temperature dependent, detachment of the phycobilisome from the chlorophyll-containing thylakoid membrane. They concluded that as the temperature is lowered there is either a complete detachment of some of the phycobilisomes or a limited detachment of all the phycobilisomes. A limited detachment of all the phycobilisomes, so as to lower the transfer effeciency, would increase the transfer time. Since the opposite is observed in the present work, this possibility may be ruled out. (As the temperature is lowered in the physiological range, and in the absence of DCMU, the time required for energy transfer to chlorophyll [690 nm] decreases.) A complete detachment of some of the phycobilisomes would not modify the transfer time, provided all attachments between the phycobilisomes and the chlorophyll-containing thylakoid are the same. Since we observe a decrease in transfer time as the temperature is lowered, this would indicate a closer distance or attachment between phycobilisome and chlorophyll. To make our observations consistant with those of Schrieber et al. (1979) we suggest that the attachments between the phycobilisome and the thylakoid are not all the same. As the temperature is lowered, there may be complete detachment of the "more" distant or poorly oriented phycobilisomes. On the other hand, the population of tightly attached phycobilisomes, which have a short distance to the chlorophyll, remains attached to the chlorophyll containing thylakoid. The net result would be a decrease in the rise time for fluorescence, accompanied by a decrease in transfer efficiency. Apparently, lowering the temperature eliminates or weeds out those phycobilisomes that transfer energy to chlorophyll at a slow rate. In agreement with this suggestion, Gantt et al. (1979) reported that low temperature (4°–10°C) results in uncoupling of energy transfer and partial dissociation of isolated phycobilisomes. Measurement of energy transfer by picosecond absorption spectroscopy might provide useful insight (Kobayashi et al. 1979).

Measurements of difference absorption spectra of an organism at different temperatures show significant changes in spectra (Brody and Singhal, 1979). For example, decreasing the temperature, to 0°C results in a decrease of a long wavelength (700 nm) absorption band; increasing the temperature results in an increase in absorption of the long wavelength absorption band. (We have recently observed discrete, temperature dependent changes in the absorption spectrum of the phycobilins.) The magnitudes of these reversible temperature-dependent spectral changes are 1% or less of the total absorption. These spectral changes, though small, could reflect important changes in the distance and orientation between molecules, which in turn could effect the rate of energy transfer.

In the present work we report that the fluorescence maxima for B-phycoerythrin and chlorophyll (690 nm) in *P. cruentum*, at 23°C, are separated in time by 28 ps. Porter et al. (1978) reported a time difference of ~40 ps for the same pigment system. The difference between the two values might arise from differences in the experimental conditions such as temperature of the algae during the measurements, age and culture conditions of the algae, or the presence of 10^{-4} M DCMU in Porter's work. As organisms age, the content and composition of pigments change. This fact could modify the distances and orientation between molecules.

It is observed that DCMU modifies the position of the fluorescence peaks differently at 0° and 37°C. With *P. cruentum*, measured at 0°C, the differences in time between the phycoerythrin and chlorophyll (690 nm) fluorescence maxima is 26 ps. With *A. nidulans* at 0°C, the difference in time between phycocyanin and chlorophyll (690 nm) is 16 ps. When 10^{-4} M DCMU is added, the times between the fluorescence maxima increase to 40 and 24 ps, respectively. At 37°C the difference in time between the fluorescences maxima of phycoerythrin and chlorophyll (690 nm) is decreased upon addition of DCMU (see Table II). The position of a fluorescence peak is effected by both the rate of energy transfer and the rate of fluorescence decay. Presumably the effect of DCMU on the fluorescence life time of chlorophyll (and phycobilin) is the same at the two temperatures under consideration; with this set of conditions the position of the fluorescence peak would be effected primarily by the rate of energy transfer. If DCMU were simply closing the Photosystem II reaction centers, it should only change the fluorescence lifetime (and yield) of chlorophyll and not the rate at which energy is transferred to chlorophyll. Consequently, it appears that DCMU is effectively increasing the distance between chlorophyll and phycobiliprotein at 0°C (see Tables I and II). At 37°C, on the other hand, DCMU appears to effectively decrease the distance between chlorophyll and phycobiliprotein. The difference in the effect of DCMU on the transfer time at the two temperatures might arise from a temperature induced modification of the lipid phase of the thylakoid, and of the attachment between the phycobilisome and the chlorophyll containing thylakoid.

In general, energy transfer in *A. nidulans* and *P. cruentum* seems to follow a sequential transfer to molecules with lower energy. A striking exception is transfer to the molecular species that gives rise to fluorescence at 715 nm. The time dependent emission at 715 nm varies with temperature and the presence of DCMU. It is of interest to consider the origin of the emission at 715 nm. Spectrally, it might originate from Photosystem II pigment, an aggregated form of chlorophyll, or a phycobilin protein. All of these molecules could have a fluorescence component at 715 nm. If the 715-nm emission were simply a fluorescent component of a molecular species that has a fluorescence maximum at another wavelength, then the 715-nm fluorescence would be expected to coincide in time with the main emission maximum from that molecule. Since the time dependence of the emission at 715 nm is neither consistant with the emission from Photosystem II (at 690 nm) nor with any of the phycobilins, it appears that the 715 nm emission may originate from an aggregated form of chlorophyll (or Photosystem I). Emission at 715 nm from an aggregated form of chlorophyll might arise in either of two ways. Energy absorbed by phycoerythrin could be transferred directly to aggregated chlorophyll. In *A. nidulans*, Csatorday et al. (1978) presented evidence for direct energy transfer between phycocyanin and chlorophyll *a*, bypassing the sequential transfer via allophycocyanin and allophycocyanin B. This might account for those cases when emission at 715 nm preceeds emission from phycocyanin (Fig. 2) or from chlorophyll (690 nm) (Fig. 6). Whether or not caratenoids are involved in the absorption and transfer of energy is not known. It is also possible that some of the excitation energy at 530 nm is absorbed directly by an aggregated form of chlorophyll, thus directly giving rise to the fluorescence at 715 nm.

Dr. Brody would like to thank Prof. G. Porter for making this work possible by providing encouragement, space, facilities, and the gracious hospitality at The Royal Institution in London.

Received for publication 10 June 1980 and in revised form 12 January 1981.

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