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Partial uncoupling of energy transfer from phycoerythrin in the marine cyanobacterium *Synechococcus* sp. WH7803

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Time-resolved fluorescence spectroscopy of the marine cyanobacterium *Synechococcus* sp. WH7803 showed that a significant fraction of the biliprotein phycoerythrin did not transfer its excitation energy to the photosynthetic reaction centres when the organism had been cultured in non-limiting light conditions and with excess nitrogen supplied as nitrate. We consider that this partial decoupling of energy transfer shows the capacity that this organism has to use a portion of the phycoerythrin as a nitrogen reserve, without the corresponding photo-oxidative damage that could occur from having a larger functional antenna than is required by photosynthesis.

Introduction

Picoplanktonic cyanobacteria of less than 2.0 μm diameter, such as *Synechococcus* sp., make a substantial contribution to primary productivity throughout the world's oceans [1–3]. Most obligatory marine isolates of these small autotrophs synthesize the biliprotein phycoerythrin (PE) as a major component of their light-harvesting apparatus [4]. It has been suggested that in the oceanic isolate *Synechococcus* sp. WH7803, PE not only is a light-harvesting pigment but also a functionally distinct nitrogen store [5]. Evidence supporting this was obtained from the comparison of *Synechococcus* cells grown in continuous culture under either nitrogen-sufficient or nitrogen-limited conditions. The mobilisation of PE accumulated by nitrogen-replete cells was sufficient to maintain the growth rate during short-term periods of nitrogen starvation; however, interruption of the supply to nitrogen-limited cells resulted in an immediate decline in growth rate, resulting in a significantly lower biomass at stationary phase [5]. The nitrogen-replete cells had a higher PE content than nitrogen-limited cells (21.9 μg/unit of biomass against 16.3 μg/unit) and the higher PE:PC ratio of 21.6 to 12.5. Two complementary observations suggest that although the nitrogen-sufficient cells had this higher PE content, a fraction of the light absorbed by PE was not transferred to the reaction centres. Firstly, preferential excitation of PE with a series of 540 nm flashes resulted in the turnover of a similar number of PS II reaction centres in both high- and low-nitrogen grown cells, although the PE cell concentration was about 40% higher in the nitrogen-replete cells. Secondly, from measurements not only of the relative steady-state fluorescence of PE in cells treated with 50% glycerol, which uncouples energy transfer from PE to chlorophyll and hence the reaction centre [5], but also of untreated cells, a substantial but unquantified, fraction of the excitation was lost as fluorescence [5].

This conclusion was questioned by Yeh et al. [6], who measured PE fluorescence decays in intact cells and a freshwater isolate as a control. In *Synechococcus* sp. WH7803 Yeh et al. [6] found that the proportion of PE not coupled to the reaction centres did not exceed 2%. They estimated that the results of Wyman et al. [5] could be the result of small, about 3%, changes in the cell content of PE uncoupled from energy transfer, although it has been pointed out that they produced their experimental material under different growth conditions [7].

This paper reports picosecond time-resolved measurements of PE fluorescence in intact *Synechococcus*

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Abbreviations: PE, phycoerythrin; PC, phycocyanin.

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sp. WH7803 cells grown under precisely defined conditions. We find that while less than 2 to 4% is uncoupled in low-nitrogen growth conditions, this rises to about 12% when the nitrogen supply is sufficient.

Materials and Methods

Organism

Synechococcus sp. WH7803, formerly known as DC2, was obtained from Dr. J. Waterbury, Woods Hole Oceanographic Institution, USA, and was grown in an artificial sea water medium as previously described [5]. Experimental material was grown in continuous culture at an incident photon flux density (irradiance) of 120 μE m^{-2} s^{-1}. Sodium nitrate reservoir concentrations were 750 and 19.5 mg litre^{-1} for nitrogen-replete and nitrogen-limited cultures, respectively. At steady state, specific growth rates were 0.052 h^{-1} for each treatment. The optimum photon flux density for growth was about 25 μE m^{-2} s^{-1}, but cultures were routinely grown in excess of this because it was critical for the detection of uncoupled PE that the organism was not light-limited during growth.

Time resolved fluorescence

Fluorescence decays were measured both by a streak-camera and by single photon counting. In the streak-camera experiments, an amplified laser pulse of 1 ps duration was used to generate a continuum in a water/deuterium oxide mixture [8]. Samples were excited at 548 nm with 1 ps pulses by isolating a portion of the continuum with an interference filter of 9 nm bandpass at 50% transmission. Fluorescence was detected, at right angles to excitation through a similar filter, transmitting at 578 nm for PE emission. An Imacon 500 streak-camera (Hadlands) was used which was coupled to an optical multi-channel analyser (Princeton Applied Research OMA 1254/1216) by two f 1.2 lenses and data were read from the OMA by a microcomputer. The slit width-limited instrument response function was 10 ps fwhm.

A cavity-dumped dye laser operating at 540 nm was used for the single photon counting experiments [9]. An XP 2020Q photomultiplier detected the fluorescence, the instrument response function was 360 ps fwhm. The fluorescence decays were analysed using the equation:

$$I(t) = a(f \exp(-t/\tau_1) + (1-f)\exp(-t/\tau_2)) \quad (1)$$

and a non-linear least-squares method was used to estimate the parameters [9]. Convolution with the instrument function was necessary only for the photon-counting experiments because of their poorer time resolution compared to the streak-camera. Consequently, the τ_2 decay times in Table I are shorter from streak-camera than photon-counting measurements. The greater signal-to-noise ratio of photon counting, however, enables more accurate determination of the fraction of any long-lived component.

Results and Discussion

The excitation (548 nm) and emission (578 nm) wavelengths used were close to the in vivo maxima for

TABLE I

Phycoerythrin excited state lifetimes and yields from nitrogen sufficient and nitrogen-limited cultures of the cyanobacterium Synechococcus sp. WH7803

The lifetimes are τ_1 and τ_2; the fraction uncoupled, f, see Eqn. 1.

	τ_1 (ps)	f (%)	τ_2 (ps)	ϕ [a]	Ratio [b] $(1-f)/f$	Ratio [c] $\tau_1/\tau_2 \cdot (1-f)/f$
N-limited: culture 1						
SC [d]	1377	1.9	83	0.76	51.6	3.1
SPC [d]	1340	3.8	130	0.71	25.3	2.5
N sufficient: culture 2						
SC	1276	13.5	110	0.36	6.4	0.55
SPC	1325	12.2	161	0.46	7.2	0.87
N-sufficient: culture 3						
SC	1250	6.3	90	0.51	14.9	1.1
SPC	1230	15.3	230	0.51	5.5	1.0
N sufficient: culture 4						
SC	1182	11.0	81	0.36	8.1	0.55
+ 50% glycerol, SC	1220	100	–	–	–	–
N-sufficient: Yeh et al. [6]	2010	2	94	0.79	49	2.3

[a] Coupled yield: $\phi = \tau_2(1-f)/(\tau_1 f + \tau_2(1-f))$

[b] Ratio of coupled to uncoupled pigments from lifetimes.

[c] Ratio of coupled to uncoupled pigments from yields.

[d] SC, streak camera; SPC, single photon counting.

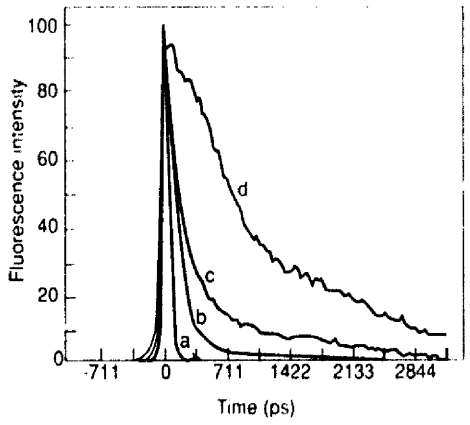

Fig. 1. Time-resolved phycoerythrin emission from whole cells of *Synechococcus* sp. WH7803. Data were collected on suspensions of 10^{13} cells dm^{-3} in growth media at 293 K and circulated through a 5 mm path-length flow cell using a peristaltic pump, to avoid overexcitation of the sample. Trace (a), Excitation laser profile; trace (b), nitrogen-limited cells (culture 1); trace (c), nitrogen-sufficient cells (culture 2); trace (d), culture 2 in 50% glycerol. Parameters from the analysis of these decays are shown in the table.

PE absorption (542 nm) and fluorescence (565 nm). The fluorescence decay results are shown in Table I. In each sample there are fast and slow decaying components of about 100 ps and about 1300 ps, respectively. When 50% glycerol is added to intact cells all PE emission has a single lifetime of 1200 ps (Table I); thus, we attribute the long decay (τ_1) to energetically uncoupled PE and the other lifetime to coupled PE which is active in photosynthesis. The shorter lifetime is in good agreement with those determined previously for PE in intact cells of *Synechococcus* WH7803 [6]. The longer lifetime is shorter than that observed by Yeh et al. [6], who reported a lifetime for uncoupled PE that corresponded with the lifetime for free PE complexes [10]. However, recent studies of a cyanobacterial PS II deletion mutant [11] have shown that the lifetime of phycobilisomes lacking energy transfer to PS II is shorter than that observed in isolated phycobilisomes, although the fluorescence quenching mechanism is unknown.

We observed sequential energy transfer by additionally measuring the emission from PC at 645 nm and also emission above 670 nm. At 645 nm the PC rise-time ($1/e$) was 38 ps in both nitrogen-sufficient and nitrogen-limited cultures. The PC decayed with a 100 ps lifetime. The faster rise-time for PC than PE decay is observed in other phycobilisomes [12] and is due to the spatial distribution of pigments and the long range of the energy-transfer process. A rise-time for emission detected above 670 nm of 50 ps and decay time of 180 ps was also observed. Because of overlapping emissions both *allo*-phycocyanin and chlorophyll fluorescence could be being observed at these wavelengths.

A direct measure of the the yield of coupled PE complexes is given by

$$\phi = \tau_2(1-f)/(\tau_2(1-f)+\tau_1 f)$$

and shown in Table I. The ratio of coupled to uncoupled PE can also be estimated and can be based either on lifetimes or yields. The ratio obtained using lifetimes is $(1-f)/f$, but that from yields is $(\tau_2/\tau_1)(1-f)/f$, which is less than the corresponding data based on lifetimes (Table I) because of the ratio τ_2/τ_1. Both these ratios and ϕ are smaller in the nitrogen-sufficient cultures than in the nitrogen-limited ones. In the nitrogen-limited cultures the fraction of uncoupled PE, f, was always below 4%; in the nitrogen-sufficient cultures this fraction has an average of 11.7%.

In samples taken from continuous cultures incubated under non-limiting photon flux densities, Wyman et al. [5] measured a decrease in the relative light-harvesting or 'coupling efficiency' of PE from about 10 in nitrogen-limited cells to between 5.6 and 6 in nitrogen-replete cells, i.e., a decrease of between 1.7- and 1.8 times. The corresponding decrease reported here from the ratio of ϕ values is 1.5- to 2-times. The results in Table I clearly show that there is a 3- to 5-fold increase in the amount of uncoupled PE in nitrogen-sufficient cells to a value of about 12%. This is consistent with the interpretation that PE is accumulated for functions other than light-harvesting when external combined nitrogen is readily available and light is non-limiting. Since the difference in PE content of nitrogen-replete and nitrogen-limited cells is considerable, both coupled and uncoupled PE must be mobilised during prolonged nitrogen starvation to supply sufficient nitrogen for sustained growth [5].

Many photosynthetic organisms, including cyanobacteria, possess mechanisms which rapidly alter the distribution of excitation energy between Photosystems I and II in response to changes in illumination conditions. Although these state transitions affect fluorescence from the phycobilisomes and chlorophyll, they have been recently reported [15] to affect the apparent emission amplitude from the terminal emitter of the phycobilisome (680–685 nm) and the fluorescence from Photosystem II, also at 680 nm. The experiments reported here preferentially excite PE and measure close to PE peak emission. Thus, we do not believe that our results can be explained by an alteration in the distribution of excitation energy between the reaction centres. Additionally, neither the rise-times or decay-times of emission from other pigments than PE were altered by culture conditions, suggesting that the nitrogen availability has no effect on energy transfer from coupled PE.

The ratio of in vivo PE steady-state emission intensity, I, in the presence to that in the absence of glycerol, which completely uncoupled the energy trans-

fer between PE and the reaction centre, has been called the 'coupling efficiency' [5] and is defined as

$$\eta = \frac{I_{0,u}(\text{glycerol})}{I_{0,u}(\text{no glycerol})}$$

The coupling efficiency can also be expressed as

$$\eta = \frac{\tau_u}{(f_u\tau_u + (1 - f_u)\tau_c)}$$

where τ_c and τ_u are the lifetimes coupled and uncoupled, respectively, and f_u is the fraction of PE uncoupled; η can take values between 1 and τ_u/τ_c. Using η values from steady-state experiments, 5.5 to 6 in nitrogen-replete cells to approx. 10 in nitrogen-limited cells, and the lifetimes, τ_u = 1300 ps, τ_c = 100 ps (Table I), the fraction uncoupled, f_u, is 2.5% in nitrogen-limited cells and 9.7% in nitrogen-replete cells. These values are consistent with f, the fraction uncoupled, measured directly from fluorescence decays, Table I, and with the results of Yeh et al. [6], who reported that 2% of PE was uncoupled. The lowest value of η which we have observed from growing cultures of *Synechococcus* is 4, which corresponds to 18.7% uncoupled PE.

Cyanobacteria are unique among microorganisms in that they may have two macromolecular reserves of nitrogen: the phycobilisomes and the storage polymer cyanophycin [14,15]. Recently, it has been shown that the cyanophycin was absent from *Synechococcus*, a related strain WH8018 and several other members of the genus *Synechococcus* [16]. Thus, phycobiliproteins are the only recognised macromolecular reserves of nitrogen in these organisms and they can account for a considerable proportion of cell protein, frequently 20 to 40%.

The data given here show clearly that there is a 3- to 5-fold increase in the amount of uncoupled PE in nitrogen-replete cells of *Synechococcus* sp. WH7803 to a level of about 12%. This finding is consistent with our original interpretation [5] that PE is accumulated by *Synechococcus* for a function other than that of light-harvesting when an appropriate supply of nitrogen is readily available and light is non-limiting. We suggest that the accumulation of energetically uncoupled PE in *Synechococcus* sp. WH7803 cells reflects an enhanced capacity of this organism to store nitrogen in this form. The uncoupling of PC as a result of photobleaching in *Anabaena variabilis* [17] is quite distinct from the partial uncoupling of PE described here; cultures of *Synechococcus* WH7803 were analysed after exponential growth at photon flux densities that did not cause photo-inhibition or photo-bleaching.

Marine *Synechococcus* sp. are the dominant primary producers in the upper layers of the oligotrophic ocean [4], where the nitrogen availability is often considered to limit growth [18]. There is clearly a considerable competitive advantage in having the ability to store the nutrient which is in shortest supply, particularly if this supply is episodic [19]. Since there is a finite amount of energy which can be delivered to an individual photosynthetic reaction centre without photo-oxidative damage, we consider that the capacity to store nitrogen in the form of PE is enhanced by regulation of the degree of energetic uncoupling of that pigment from the reaction centres.

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