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Rapid Report

Photosynthetic energy storage in cyanobacterial cells adapted to light-states 1 and 2. A laser-induced optoacoustic study

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We have used laser-induced optoacoustic spectroscopy to investigate photosynthetic energy storage during the first 1.4 μs after a laser excitation flash in cells of the cyanobacterium *Synechococcus* 6301 adapted to light-states 1 and 2. We find no decrease in energy storage on transition to light-state 2, indicating that energy diverted away from Photosystem II is not quenched but may be transferred to Photosystem I.

In common with other photosynthetic organisms [1], cyanobacteria possess the ability to modify the function of their light-harvesting apparatus as a rapid response to changes in the intensity and spectral quality of actinic light [2]. These changes are known as state 1–state 2 transitions. The functional effects of state transitions in phycobilisome-containing organisms have been investigated mainly by various forms of fluorescence spectroscopy and have been the subject of recent debate [3]. Based on fluorescence data, which report mainly on Photosystem II, two main models have been proposed:

(a) State transitions change the proportion of PS II reaction centres that are coupled to the phycobilisomes [4–6].

(b) State transitions change the extent of spillover (excitation energy transfer from PS II to PS I) [7,8].

Recent measurements of the kinetics of fluorescence decay in cyanobacterial cells on a picosecond timescale support model (a) rather than model (b), since the transition to state 2 was found to decrease the amplitudes of the fluorescence decay components associated with PS II without changing their lifetimes [5,9]. However, state transitions had no significant effect on the kinetics of fluorescence decay from the terminal emitter chromophores of the phycobilisomes, which have a lifetime of 180–200 ps in both states [5,9]. This indicates that there is an alternative acceptor for excitation energy from phycobilisomes when PS II is decoupled in state 2. Two possible candidates for this acceptor are:

(a) A non-radiative quenching process. In this case, the energy which is not transferred to PS II in light-state 2 is simply converted into heat.

(b) Photosystem I. Direct energy transfer from phycobilisomes to PS I could not be detected by time-resolved fluorescence spectroscopy [5,9]. Such an energy-transfer process would be very difficult to detect because of the rapid fluorescence decay from PS I: energy would be transferred from phycobilisomes to PS I with a rate constant of about 5 ns^{-1} and would then decay in the PS I chlorophyll antenna with a faster rate constant of about 25 ns^{-1} [9]. Energy transfer from the phycobilisomes would not create a significant additional population of excitons in the PS I core complex and the amplitudes of the fluorescence components resulting from energy transfer to PS I would therefore be extremely small.

A technique other than fluorescence spectroscopy is therefore required to distinguish between these two possible acceptors. Here, we report measurements of heat evolution and energy storage in cyanobacterial cells adapted to light-states 1 and 2. We have used

Abbreviations: Chl, chlorophyll; E_0, laser energy fluence per pulse; EG, ethylene glycol; H, optoacoustic signal amplitude; H_N, H/E_0; LIOAS, laser-induced optoacoustic spectroscopy; PS, Photosystem; RC, reaction centre.

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laser-induced optoacoustic spectroscopy (LIOAS) [10,11] to determine the heat evolved by the cells in the first 1.4 μs following a laser pulse.

The application of LIOAS to photosynthetic systems has previously been described in detail [12,13]. Laser pulses (15 ns fwhm) were generated at a repetition rate of 1 Hz by an excimer-pumped dye laser which was tuned to either 630 nm or 690 nm. The diameter of the laser beam was 2 mm and its intensity was controlled with neutral density filters. The optoacoustic signals (sound-waves generated by heat evolution in the sample) were detected by two 400 kHz piezoelectric transducers (PZT ceramic, Vernitron) attached to opposite sides of a 1 cm flow cuvette [12]. The signals were added and fed into a computer-controlled LS173/μVAX transient recorder (Biomation 4500, Gould). At the low-energy limit, 100 signals were averaged; this was decreased to 10 signals at higher excitation energies.

The sample consisted of cells of the cyanobacterium *Synechococcus* 6301 (*Anacystis nidulans*) grown as previously described [5]. The concentration of Chl *a* in the cells was determined using the formulae of Myers et al. [14] and the cells were diluted to a concentration of 5 μM Chl *a* in 70% growth medium and 30% ethylene glycol (EG). Since EG possesses a higher cubic expansion coefficient, its addition increased the size of the optoacoustic signal [12,13]. The presence of 30% EG had no effect on the fluorescence yield of the cells or on their ability to perform state 1–state 2 transitions. The absorbance of the sample suspension was 0.46 at 630 nm and 0.27 at 690 nm (corrected for light-scattering). 430 ml of suspension was used for each measurement and was pumped through the flow cuvette at 100 ml min^{-1}, sufficiently fast to ensure that the cells were exposed to only one laser flash in each cycle. The delay between the illuminated reservoir and the measurement was about 3 s. During the measurement, the bulk of the suspension was kept in a stirred reservoir at room temperature (about 20 °C). For state 2, the cells were dark-adapted. For light-state 1, the cells were illuminated with far-red light defined by a Schott RG685 filter at an irradiance of about 0.5 W m^{-2}.

Adaptation to state 1 or to state 2 was monitored by measuring the steady-state fluorescence yield of the cells at the flow cuvette with a Modulated Fluorescence Measurement System (Hansatech, King's Lynn, U.K.) as previously described [5]. Excitation was with yellow light (maximum at 583 nm) predominantly absorbed by phycocyanin. Far-red light induced a reversible increase of about 12% in the steady state-state fluorescence yield of the cells which was complete in, about 2 min. This is typical for the state 1 transition in this cyanobacterium [15]. Since most of the fluorescence observed with phycocyanin-absorbed excitation light is the invariant contribution from phycobilisomes rather than from PS II when PS II reaction centres are open [4], the actual increase in PS II fluorescence must be much larger than 12%.

The size of the optoacoustic signal indicates the amount of heat released integrated over the acoustic transit time across the diameter of the laser beam, in this case 1.4 μs [10,12,13]. This time includes heat evolution due to light-harvesting processes and early electron transfer events in the RC, but excludes oxygen evolution and the slower electron transport steps requiring diffusion of electron carriers [12,13]. This is the major advantage of LIOAS over conventional photoacoustic spectroscopy with amplitude-modulated sources [10]. To quantify the measurement we have used the signal from a calorimetric reference (a solution of $CuCl_2$ in 30% EG with the same absorbance ($\pm$0.01) as the cell suspension). This solution releases all the absorbed energy as heat within the integration time of 1.4 μs, allowing a calculation of the fraction of absorbed energy stored by the cell suspension.

When applying LIOAS to photosynthetic systems it is important to use low excitation energy fluences corresponding to one absorbed photon per RC or less. For higher excitation fluences the RCs are no longer photosynthetically active, so that a much higher proportion of the absorbed energy is released as heat [12,13]. We have used a range of excitation energies from 250 nJ/pulse to about 1 mJ/pulse. Assuming the antenna sizes and PS II/PS I stoichiometry reported by Myers et al. [14], the former corresponds to an average of about 0.5 absorbed photons per RC (PS I and PS II) at 630 nm. However, the concentration of excitons in the reaction centres actually bound to phycobilisomes will be somewhat higher than this.

Fig. 1 shows the results of LIOAS measurements for *Synechococcus* 6301 cells adapted to light-states 1 and 2 with excitation at 630 nm, absorbed predominantly by phycocyanin [16]. The amplitude (H) of the signal has been normalised to the excitation energy (E_0) to give a

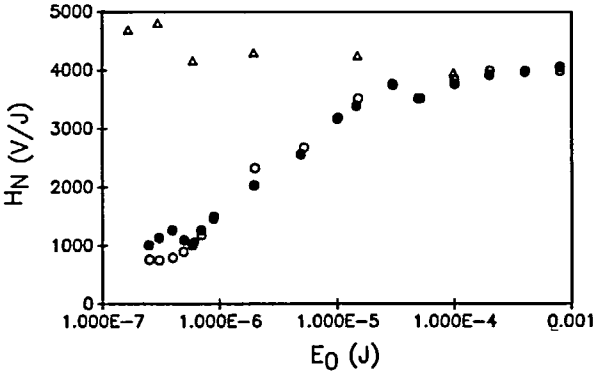

Fig. 1. Energy-normalised LIOAS signals with 630 nm excitation for *Synechococcus* 6301 cells adapted to states 1 (•) and 2 (○) and for a $CuCl_2$ solution with same absorbance (△). Note: the irregularities in the values for the reference reflect instrumental artefacts.

measure (H_N) of the extent of conversion of the excitation pulse to heat. At low excitation energies (< 1 μJ) H_N is low and increases little with increasing excitation energy; this indicates that most RCs remain open [12,13]. As the excitation energy is increased, H_N rises steeply due to the closure of RCs, finally reaching the reference level. The shape of the saturation curve is comparable to that observed by Nitsch et al. for isolated RC particles and predicted by a multiple-hit Poisson distribution model [12,13]. However, since whole cells contain several populations of RCs differing in antenna size and composition, the saturation curve observed in whole cells probably represents the sum of several curves of the type found by Nitsch et al [12,13]. The only significant difference between the LIOAS signals for cells adapted to state 1 and to state 2 is at the low-energy limit, where H_N is perhaps slightly greater for cells in state 1 (Fig. 1).

Fig. 2 shows the results with $\lambda_{exc} = 690$ nm (light absorbed predominantly by the Chl *a* antenna of PS I). Under these conditions the LIOAS signal was too small to be detected at the low-energy limit for open RCs. This indicates a very high efficiency of storage of absorbed energy with 690 nm excitation, higher than with 630 nm excitation (Table I). This may reflect two factors:

(a) PS I is more photochemically efficient (i.e., it stores a larger fraction of the absorbed energy) than PS II, as demonstrated by LIOAS measurements on isolated reaction centres [12], or

(b) with 630 nm excitation, excitation energy is converted to heat as photons absorbed by phycocyanin are transferred to pigments absorbing at longer wavelengths. The energy of a 690 nm photon is about 9% less than that of a 630 nm photon. Thus, with 630 nm excitation about 9% of absorbed energy will be lost in this way, even if the quantum efficiency for energy transfer to the reaction centres is 100%.

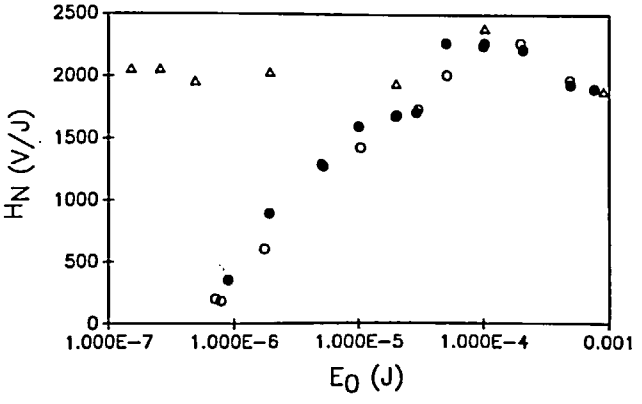

Fig. 2. Energy-normalised LIOAS signals with 690 nm excitation for *Synechococcus* 6301 cells adapted to states 1 (•) and 2 (○) and for a $CuCl_2$ solution with the same absorbance (△) (see note to Fig. 1).

TABLE I

Photosynthetic energy storage in Synechococcus 6301 cells at the low energy limit (RCs open)

The energy-normalised LIOAS signal, H_N, from the cells divided by the signal from the $CuCl_2$ standard and subtracted from 1 affords the fraction of absorbed energy stored after 1.4 μs. Data are taken from Figs. 1 and 2. With 690 nm excitation (Fig. 2) the LIOAS signal was too small to be detectable at the low-energy limit and only lower estimates of the fraction of energy stored could be obtained. Estimates of the fraction of energy stored are accurate to within about ±10%

Excitation wavelength (nm)	Light-state	H_N (V/mJ)	Fraction energy stored
630	1	1.01	0.78
630	2	0.76	0.84
690	1	< 0.35	> 0.83
690	2	< 0.20	> 0.93

With 690 nm excitation there was no detectable difference between the LIOAS signals for cells adapted to state 1 and state 2 (Fig. 2).

If the energy which is not transferred from phycobilisomes to PS II in state 2 is instead dissipated by a non-radiative quenching mechanism, as suggested in [5], we would expect a high proportion of the absorbed energy to be released as heat, even at the low-energy limit for open RCs. Our results clearly show that this is not the case. Phycocyanin-absorbed (630 nm) light is efficiently stored in both light-states, and in fact a slightly higher proportion of the absorbed energy is stored for a time longer than 1.4 μs in state 2 than in state 1 (Table I). This shows that, in state 2, energy is transferred from phycobilisomes to an acceptor other than PS II that is capable of storing energy. The most obvious candidate is PS I. We cannot, on the basis of the results reported here, distinguish between direct energy transfer from phycobilisomes to PS I and indirect energy transfer via PS II (spillover). However, picosecond time-resolved fluorescence spectra indicate that little or no change in the extent of spillover occurs during state transitions [5,9], and fluorescence induction measurements have shown that a proportion of PS II core complexes become decoupled from the phycobilisomes on transient to state 2 [6]. Taken together, these results suggest that the transition to state 2 involves the decoupling of phycobilisomes from the PS II core complexes and their subsequent coupling to PS I.

Our data support the concept that state transitions in phycobilisome-containing organisms are a mechanism for redistributing excitation energy between the photosystems, as originally proposed by Murata [17] rather than simply a mechanism for protecting PS II from exposure to high fluences. The results with 690 nm excitation (Fig. 2 and Table I) indicate that no

major energy-dissipating pathway around PS I, of the type proposed by Rehm et al. [18] was in operation under our conditions.

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