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CHANGES IN PHOTOSYNTHETIC ACTIVITY IN THE CYANOBACTERIUM *CHLOROGLOEA FRITSCHII* FOLLOWING TRANSITION FROM DARK TO LIGHT GROWTH

E. HILARY EVANS [a,*], NOEL G. CARR [a] and MICHAEL C.W. EVANS [b]

[a] *Department of Biochemistry, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, and* [b] *Department of Botany and Microbiology, University College London, London, WC1E 6BT (U.K.)*

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Summary

The cyanobacterium *Chlorogloea fritschii* loses Photosystem II activity, measured by delayed fluorescence and oxygen evolution, during dark heterotrophic growth, but retains Photosystem I, measured as light induced EPR signals. Following transition to the light, Photosystem II recovers in two stages, the first of which does not require protein synthesis. New Photosystem I reaction centres are not synthesised until after net chlorophyll synthesis has commenced. Carbon dioxide fixation recovery commences immediately, the initial rate being unaffected by chloramphenicol. The recovery of carbon dioxide fixation is not directly related to oxygen evolution rate and is only inhibited slightly by 3-(3,4-dichlorophenyl)-1,1-dimethylurea and 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone.

Introduction

Chlorogloea fritschii is a cyanobacterium (blue-green alga) which is unusual in that it can adopt heterotrophic, photoheterotrophic and photoautotrophic modes of growth [1], these being associated with differences in cell physiology and morphology [2]. When grown heterotrophically in the dark the organism loses most of its capacity for light-induced oxygen evolution [3], although it retains chlorophyll and a markedly reduced level of the accessory photopigment, phycocyanin. In the latter respect it contrasts with the cyanobacterium *Plectonema boryanum* which retains phycocyanin after dark growth [4]. The shift

* Present address: Biology Division, Preston Polytechnic, Preston, Lancashire, U.K.
Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone.

from dark heterotrophic to photoheterotrophic growth, with sucrose as carbon substrate, provides a valuable system for the study of the reacquisition of complete photosynthesis in a prokaryotic cell, and allows comparison with the well studied photosynthetic bacteria and eukaryotic microalgae.

The kinetics of the recovery of O_2 evolution by *C. fritschii* following a dark-light shift have been reported [5]. The half-recovery time is approximately 1 h. In the presence of chloramphenicol recovery is unaffected for the first hour, but is then inhibited, only half the control rate being attained. This lower rate is however, maintained for 6 to 7 h following the dark to light transition. It has therefore been suggested [5] that the recovery of oxygen evolution by dark grown *C. fritschii* proceeds in two phases, one chloramphenicol insensitive (half-recovery time about 20 min), followed by a chloramphenicol sensitive phase.

This paper describes changes in delayed fluorescence, following a dark light shift in order to compare this second parameter, associated with Photosystem II, with the oxygen evolution. In addition, activity of Photosystem I is measured, together with carbon dioxide fixation. All these results are discussed with reference to reacquisition of total photosynthetic competence by dark grown *C. fritschii.*

Methods

Chlorogloea fritschii Mitra (The Collection of Algae and Protozoa, No. 1B 1411/1, Botany School, Cambridge) was maintained in darkness for about 5 years as previously described [2] and grown for 5—6 weeks in 500 ml batches at 34°C in medium C of Kratz and Myers [6] supplemented with $NaHCO_3$ (0.1%) and sucrose (10 mM). Dark to light transitions were performed by removing aliquots from the larger culture (500 ml) to smaller sterile flasks and placing these in an illuminated orbital shaker (Gallenkamp Ltd.) containing three 30 W Grolux warm white fluorescent lamps emitting about 1030 lux, at 34°C.

Photosystem I components were estimated by EPR spectroscopy in crude membrane preparations of *C. fritschii* as previously described [5]. The concentration of the membrane fragments was normalised with respect to chlorophyll before EPR measurements were made. The relative amounts of functional Photosystem I reaction centre were estimated by measuring the size of the signal of iron-sulphur centre A (g = 1.94) induced by illumination of the sample at 20 K. This signal is quantitatively related to the extent of *P*-700 photooxidation [7,8]. Direct measurement of the *P*-700 radical is not a reliable measure of *P*-700 because of variations in EPR characteristics under different sample preparation conditions [8]. The relative amounts of total bound ferredoxin in the sample were measured as the total bound ferredoxin signal induced by illumination at room temperature in the presence of sodium dithionite. EPR spectra were recorded using a Varian E^4 spectrometer as described previously [9]. Spectra were recorded at 20 K with the following instrument settings. Frequency 9.2 GHz, power 20 mW, modulation amplitude 1 mT, scan rate 50 mT · min^{-1}.

Delayed fluorescence was measured in a Becquerel rotating sector phosphoroscope essentially similar to that of other workers [10,11]. The actinic light

source was a 55 W. Tungsten iodine lamp, and luminescence was detected using an EMI 9659 B photomultiplier. Variable delays and sampling widths were possible allowing a variety of emission times to be observed, and variable time constant and zero offset were incorporated. In all the experiments using *C. fritschii* a delay of 1 ms was used and the emission was scanned from 1 ms to 5 ms after illumination. In these experiments only, the suspension medium was Ficoll/growth medium (20 : 80, v/v) not growth medium alone, to maintain a uniform suspension of algal cells which would otherwise sediment. Other conditions for the dark-light transition were as described. A fairly dilute suspension of algae (0.1 mg dry wt./ml) were used to avoid the problem of self-quenching [11].

Carbon dioxide fixation was measured as the uptake of $H^{14}CO_3$ (0.5 μCi/ml algal culture) by *C. fritschii* (1 mg/ml dry wt. cells) into that cell fraction precipitated by 5% trichloroacetic acid. Dark grown cultures were decanted into 50 ml aliquots in sterile flasks, as stated above, under the 'standard' dark to light transition conditions. Following the transition, 1 ml samples were removed at defined intervals and mixed immediately with 10% trichloroacetic acid. After 30 min precipitated organisms were collected, washed and dried on membrane filters, and radioactivity measured using a Nuclear Chicago gas flow counter.

Ribulose biphosphate carboxylase activity was assayed as incorporation of $H^{14}CO_3$ into phosphoglycerate by a membrane-free supernatant of *C. fritschii*, essentially as described by Wishnick and Lane [12]. The cells were disrupted and fractionated as described above for the preparation of samples for EPR studies.

Chlorophyll *a* was extracted, following sonication to break cell walls, by acetone/water (80 : 20, v/v) and estimated using the extinction coefficient of Strain et al. [13]. Protein concentration of the disintegrated organism was measured by the method of Lowry et al. [14].

Radiochemicals were obtained from The Radiochemical Centre, Amersham, U.K. All reagents used were of Analar grade where possible otherwise of the highest grade available.

Results

Measurement of Photosystem II activity

The recovery of light-induced oxygen evolution by dark grown *Chlorogloea fritschii* after transference to the light was measurable within 0.5 h [5], the half maximal rate of oxygen evolution was achieved after 1.5 h. The initial very low rate of oxygen evolution increased in the presence of chloramphenicol at the same rate as the control for the first 30 min. The culture containing chloramphenicol showed a lower rate of increase for the following hour, when it reached a steady state, which was maintained for some hours.

The delayed fluorescence, of a normal light-grown culture 1 ms after illumination shows a luminescence intensity peak followed by a decay over the following milliseconds (Fig. 1a (i)). Fig. 1a (ii) shows that the level of delayed fluorescence measured in dark grown cells was very low indeed. In fact, the use of phosphoroscope to measure delayed fluorescence makes it difficult not, to a slight extent, to preilluminate the cells, and it may be that delayed fluorescence

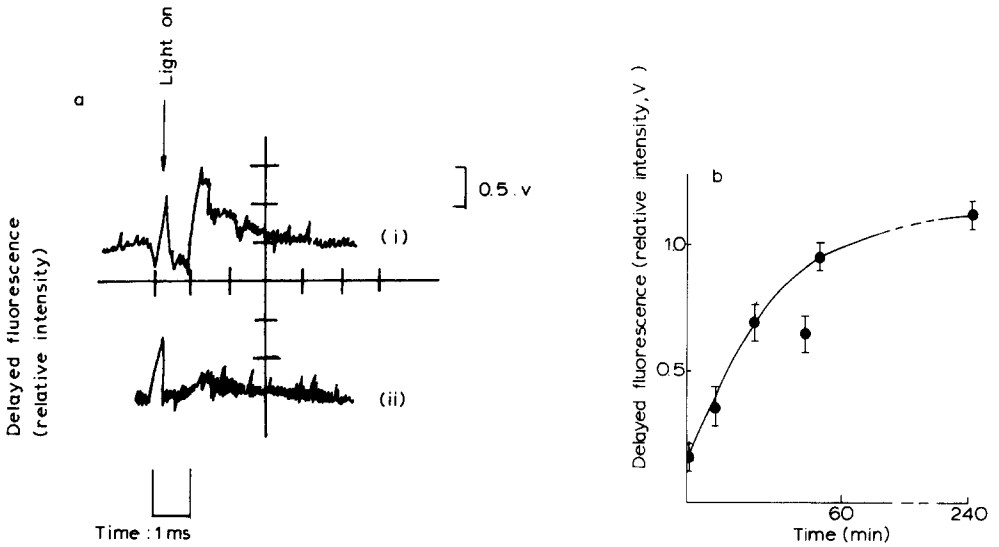

Fig. 1. (a) Delayed fluorescence measured from 1 ms to 5 ms of *C. fritschii*. Cells suspended in Ficoll/growth medium (20 : 80, v/v) to 0.1 mg/ml cell dry weight. (i) Dark grown culture after 4 h light. (ii) dark grown culture. (b) Plot of extent of delayed fluorescence 1 ms after illumination against period of time after dark-light shift. Conditions as in (a). Error bars indicate noise levels.

was negligible in dark grown cells. Following transition from the dark to light the delayed fluorescence intensity measured 1 ms after illumination, increased for 60 min and subsequently maintained this intensity (Fig. 1b). This recovery was not inhibited by chloramphenicol (20 $\mu g \cdot ml^{-1}$).

Comparison of the delayed fluorescence data (Fig. 1b) with that of light-induced oxygen evolution [5] shows a similarity in the time scale of recovery between the delayed fluorescence and oxygen evolution in the presence of chloramphenicol. Thus we may represent recovery of photosystem 2 as a two phase process; the first phase independent of protein synthesis, manifesting itself in the recovery of delayed fluorescence, and a partial recovery of light induced oxygen evolution; the second phase dependent on protein synthesis, being seen as a further increase in light-induced oxygen evolution.

Following transition of dark-grown *C. fritschii* to the light there was a constant chlorophyll *a* : protein ratio for the first 3 h, after which time the ratio began to rise, presumable due to chlorophyll synthesis [15]. It will be noted that the recovery of oxygen evolution in ref. 5 is plotted with respect to chlorophyll concentration. If expressed with respect to total protein or cell dry weight, the curve does not level off after 2.5 h, but continues to rise for a further 4 h [15], showing that synthesis of chlorophyll is concomitant with an increase in oxygen evolution. However, it must be noted that the chloramphenicol-inhibited phase of oxygen evolution recovery occurs before chlorophyll synthesis begins, and must therefore rely on synthesis of another component.

Measurement of Photosystem I activity

Fig. 2 shows the effect of illumination on the EPR spectrum of a membrane preparation from light grown *C. fritschii* cells. Oxidation of the Photosystem I

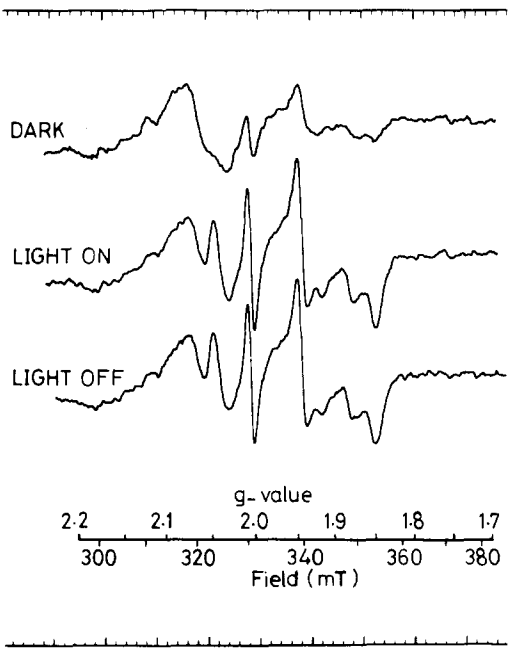

Fig. 2. EPR spectra of membrane fragments of dark grown *C. fritschii*. Fragments prepared as described. Chlorophyll concentration 1 mg/ml.

reaction centre chlorophyll (*P*-700) is observed as the appearance of a radical signal at $g = 2.00$ and reduction of Centre A of the bound iron-sulphur protein by the appearance of signals at $g = 2.05$, 1.94 and 1.86 (see ref. 16) These changes are irreversible, no further change in the spectrum is observed when the light is turned off. The Photosystem I reaction centre, observed as the photooxidation of *P*-700 and photoreduction of the bound iron-sulpur centre, was also present

TABLE I

CHANGES IN PHOTOSYSTEM I CONTENT OF *CHLOROGLOEA FRITSCHII* FOLLOWING DARK-LIGHT TRANSITIONS

Photosystem I activity measured as light induced signal at $g = 1.94$ in membrane fragments adjusted to 1 mg · ml^{-1} chlorophyll. E_1, E_2 and E_3 are three separate experiments.

Time in light (h)	Photosystem I content		
	E_1	E_2	E_3
0	29	28	78
1.0			40
1.5	20		
2.0		19	
4.5	25		
5.0		17	
15.0		14	
16.0	20		
24.0	14	14	51
48.0		20	64
72.0	38	20	

in membranes derived from dark grown cells. The photooxidation of *P*-700 and reduction of iron-sulphur centre A have been shown to be quantitatively related [7,8].

We have used the height of the light induced $g = 1.94$ signal of the iron-sulphur centre as an indicator of the relative amount of Photosystem I reaction centre in preparations from cells exposed to light for varying times. Table I shows the changes observed over the first 72 h of illumination. During the first 24 h the reaction centre to total chlorophyll ratio appears to fall. Between 24 and 48 h there is little change and between 48 and 72 h the ratio increased to about that observed in normal light grown cells. The apparent fall in reaction centre concentration is probably the result of chlorophyll synthesis during the first 24 h without reaction centre synthesis [15]. Subsequently reaction centre synthesis commences, with a return to the normal concentration in the membrane.

Measurement of carbon dioxide fixation

The change in rate of [^{14}C]bicarbonate uptake by *C. fritschii* following transition from dark to light was immediate. The level of ribulose biphosphate carboxylase activity was unchanged over the measured period (Fig. 3). It is of inter-

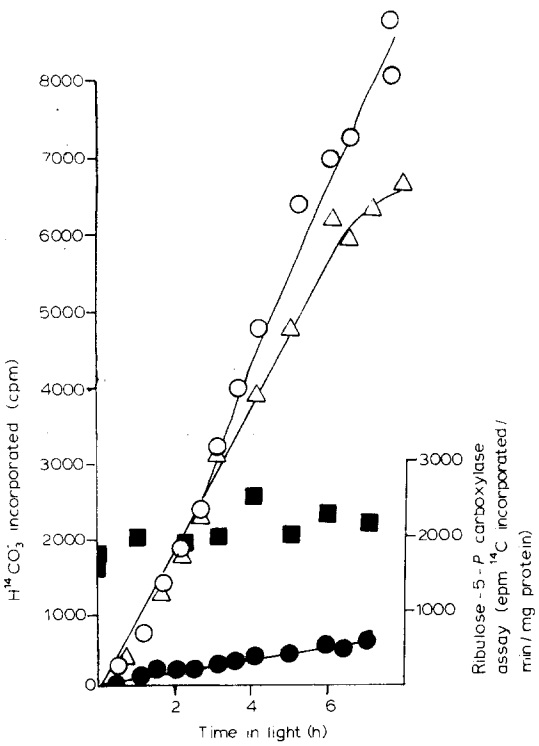

Fig. 3. CO_2 fixation by *C. fritschii* following dark-light transition. $H^{14}CO_3$ (0.2 μCi/ml) added to *C. fritschii* (1 mg/ml dry wt.). Acid precipitable counts recorded. ○, Light; ●, dark; △, light and chloramphenicol (20 mg/ml); ■, ribulose biphosphate carboxylase activity (see Methods). Photoheterotrophic culture under the same conditions incorporates approx. 40 cpm of $H^{14}CO_3$. Ribulose biphosphate carboxylase activity is similar to a photoheterotrophic culture.

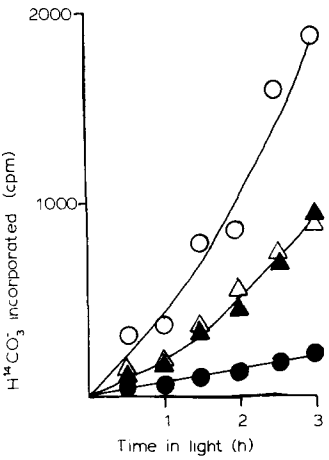

Fig. 4. The effect of DCMU and DBMIB on CO_2 fixation by *C. fritschii* following a dark-light transition. Conditions as for Fig. 3. ○, Light; △, light and DCMU (80 μm); ▲, light and DBMIB (9 μM).

est to note that *C. fritschii* fixes carbon dioxide in the dark at a significant rate.

Chloramphenicol was without effect on the [^{14}C]bicarbonate uptake rate for the first 3 h after transfer from dark to light. Uptake remained linear in the presence of chloramphenicol during the period 3—5.5 h after transition; thereafter it began to decrease following cessation of growth. In the absence of chloramphenicol an increased rate of [^{14}C]bicarbonate uptake commenced after 3 h. Thus synthesis of protein is required for the normal light-induced rate of carbon dioxide to be regained.

It may be observed that the recovery of carbon dioxide fixation does not correlate with the recovery of oxygen evolution, the carbon dioxide fixation rate remaining high when oxygen evolution rates are low. This lack of correlation is emphasised by the results shown in Fig. 4. DCMU and DBMIB, both inhibitors of non-cyclic electron transport, only inhibit the carbon dioxide fixation rate by 48—50% during the first 3 h of the recovery. In a photoheterotrophically-grown culture of *C. fritschii* DCMU or DBMIB at the concentrations employed inhibit carbon dioxide fixation by 95%.

Discussion

The parameters of photosynthesis measured during a dark to light transition of *Chlorogloea fritschii* are summarised in Table II. The recovery can be divided into three stages; an initial chloramphenicol-insensitive period, a subsequent chloramphenical-sensitive stage and a longer term recovery during which the *P*-700 to chlorophyll ratio increases and a higher growth rate commences.

The initial recovery time of Photosystem II activity was very similar to that found by Cheniae and Martin [17] using species of *Chlorella*. The presence of functional quantities of chlorophyll in dark grown *C. fritschii* was similar to that found in chloroplasts of *Chlamydomonas reinhardii* [18] and of a Gymnosperm [19] after growth in the dark, but unlike etioplasts of Angiosperms (see ref. 20). Studies on the photosynthetic bacterium, *Rhodopseudomonas capsu-*

TABLE II

TIME-SCALE OF CHANGES IN PHOTOSYNTHESIS OF *C. FRITSCHII* FOLLOWING A DARK-LIGHT TRANSITION

0 min	Photosystem II absent, Photosystem I intact	
90 min	Initial Photosystem II recovery (O_2 evolution, delayed fluorescence)	
	CO_2 fixation recovery starts	
1.5—3 h	Second phase of Photosystem II recovery chlorophyll and phycocyanin synthesis begins	} Inhibited by chloramphenicol
24—28 h	*P*-700/chlorophyll increases	
48 →	Complete recovery (?)	

lata show that development of the photosynthetic apparatus during dark growth is controlled by oxygen tension of the medium [21]. Subsequent development of the photosynthetic apparatus of *R. capsulata* following transition into the light shows that reaction centre bacteriochlorophyll is synthesised initially. The rate of synthesis decreases with time while the rate of the formation of light harvesting complex increases [22]. This is in contrast to reacquisition of normal ratios of Photosystem I reaction centre to chlorophyll *a* in *Chlorogloea fritschii*, which follows total chlorophyll *a* increase.

In conclusion, studies on cyanobacterium *C. fritschii* show some similarities to chloroplasts of green algae with respect to photosynthetic development, but differs from *Rps. capsulata*. The reacquisition of normal photosynthetic rates is not a step-wise process. The capacity for carbon dioxide fixation is present in dark grown cells and increases immediately in the light. Subsequent protein synthesis, and pigment formation, increases the rates of photosynthetic oxygen evolution and carbon dioxide fixation.

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