Biochimica et Biophysica Acta 894 (1987) 407–413
Elsevier

BBA 42680

The quenching of chlorophyll *a* fluorescence as a consequence of the transport of inorganic carbon by the cyanobacterium *Synechococcus* UTEX 625

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(Received 6 April 1987)

Key words: Chlorophyll fluorescence; Photosynthesis; Carbon transport, inorganic; Cyanobacterium; (*Synechococcus*)

The chlorophyll *a* fluorescence yield of the cyanobacterium *Synechococcus* UTEX 625 decreased upon the initiation of inorganic carbon transport. The fluorescence yield recovered upon the depletion of inorganic carbon from the medium or upon the addition of DCMU. The inhibition of photosynthetic CO_2 fixation by iodoacetamide did not prevent this reduction of fluorescence yield. Similar results were obtained for both Na^+-stimulated HCO_3^- transport and for the transport (presumably of CO_2) that is stimulated by carbonic anhydrase. A transient lowering of the fluorescence yield was also observed when cell suspensions were pulsed with CO_2. In cells not inhibited with iodoacetamide, a very close quantitative relationship existed between the net rate of O_2 evolution and the maximum extent of fluorescence quenching seen as a function of the inorganic carbon concentration. The fluorescence quenching, however, was not due to CO_2 fixation but rather to the transport of inorganic carbon or the accumulation of the internal pool of inorganic carbon. If quenching is due to the latter it is not surprising that the extent of quenching corresponds to the maximum rate of photosynthesis as the rate of photosynthesis also depends on the size of the internal pool. The results with DCMU suggest that the quenching is Q quenching and transport must provide a mechanism for the oxidation of Q other than CO_2 fixation.

Introduction

Cyanobacteria actively accumulate inorganic carbon to high concentrations within the cells due to the operation of one or more transport systems for inorganic carbon [1–7]. The elevated CO_2 concentration in the cells maintains photosynthesis even when the ambient CO_2 concentration falls far below the level required for significant activity of the CO_2-fixing enzyme ribulose bisphosphate carboxylase/oxygenase [1–9] and may also account for the reduced rate of photorespiration [10] commonly observed in such cells.

Cyanobacteria grown under carbon-limited conditions have a very high affinity for inorganic carbon [1,2,5,7–9] and can be readily taken to a very low CO_2-compensation point [10,11]. At that point, at pH 8.0, CO_2-dependent photosynthesis can be initiated by the addition of carbonic anhydrase [13,14] and HCO_3^--dependent photosynthesis can be initiated by the addition of HCO_3^- in the presence of Na^+ [13].

The active inward transport of inorganic carbon across the cyanobacterial plasmalemma is probably the major solute flux in these cells with estimated rates as high as 3800 μmol per mg chlorophyll per h [2]. The energy requirement for this transport would seem to be supplied by ATP

Abbreviations: BTP, 1,3-bis(Tris(hydroxymethyl)methylamino)propane; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; F_v, variable fluorescence.

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produced from cyclic photophosphorylation [6,15,16] and one might expect changes in the operation of the photosynthetic electron-transport chain when inorganic carbon transport is initiated. Chlorophyll *a* fluorescence from Photosystem II can be a useful in vivo probe of the electron-transport system [17–19] and leaves and isolated chloroplasts show predictable changes in fluorescence when the rate of CO_2 fixation is altered [20–28]. Similar studies have not been reported for cyanobacteria and in this paper we describe the changes in fluorescence yield of chlorophyll *a* that occur when inorganic carbon transport is initiated in *Synechococcus* UTEX 625 under various conditions.

Materials and Methods

Synechococcus UTEX 625, also known as *Synechococcus leopoliensis* and *Anacystis nidulans* [29], was grown with air bubbling as previously described [14]. For experimental use, cells were washed three times by centrifugation in an Eppendorf 5414 microfuge (15000 × *g* for 1 min) in 50 mM BTP/46 mM HCl at pH 8.0. The buffer was prepared as previously described [9] and contained only low levels of inorganic carbon (about 15 μM) and Na^+ (about 5 μM). Cells grown and prepared in this way require the addition of either Na^+ or carbonic anhydrase in order to transport inorganic carbon effectively at low concentrations [13]. NaCl was added to yield a final concentration of 20 mM and the carbonic anhydrase (Sigma, 2500 units per mg protein) to 25 $\mu g \cdot ml^{-1}$.

The simultaneous measurements of photosynthetic O_2 evolution and fluorescence yield were carried out with 1.5 ml cell suspensions (N_2 purged) in a Hansatech DW2 unit. The design and operation of the Hansatech DW2 unit is similar to that of the DW1 unit described by Delieu and Walker [30], except that the cell suspension is separated from the Clark-type Pt | Ag-$AgCl_2$ electrode by only a Teflon membrane and it can be stirred continuously with a small magnetic flea. The cells were illuminated with a slide projector with a Sylvania quartz-halogen tungsten lamp (300 W, Model ELH) powered by a Sola constant voltage transformer operating at 115 V. In the light beam were placed a Calflex C heat filter, a 605 nm Balzers B-40 interference filter (with a measured half-band width of 27 nm) and a curtain-type shutter from a 35 mm camera. The photon flux density incident upon the surface of the cuvette was 140 $\mu E \cdot m^{-2} \cdot s^{-1}$, measured with a LiCor light meter with quantum sensor.

The photodiode used to measure fluorescence emission was protected by a Balzers infrared interference filter (with measured transmission of 5% at 700 nm and 50% at 724 nm). Amplified outputs from the O_2 electrode and the photodiode were recorded on a Fisher Recordall Series 5000 chart recorder. The half-time response of the O_2 measuring system (measured by the injection of small volumes of O_2-saturated ethanol) was about 4 s. The half-time response for changes in fluorescence yield was limited by the response time of the recorder (about 0.4 s full scale deflection). The temperature of the stirred cell suspensions was maintained at 30° C. The chlorophyll concentration in the cell suspensions was between 6 and 8 μg/ml.

Fluorescence emission spectra were measured at room temperature (about 22° C) with a Tracor-Northern TN6100 intensified (60000 times) photodiode array containing 1024 photodiodes [31]. Light from a 150 W xenon arc lamp was passed through the excitation monochromator (Oriel 7240) to yield light with a peak wavelength of 590 nm and a half-band width of 37 nm. The beam was focussed onto the surface of a 1 cm Suprasil fluorescence cell (incident photon flux density = 120 $\mu E \cdot m^{-2} \cdot s^{-1}$) so that a 1 ml volume of cell suspension was evenly illuminated. Light emitted from the sample at an angle of about 125° from the illuminating beam was passed through the analyzing monochromator (Jarrel-Ash JA82-486) with the entrance slit covered by an Oriel N-63 sharp-cut-on filter. Signals were detected and analyzed as described by Pottier et al. [31].

Cells were grown and washed as usual and were placed into the fluorescence cell with N_2-purging. The head-space was flushed with N_2 and the fluorescence cell was tightly stoppered to prevent the entry of atmospheric CO_2. Within 5 min the fluorescence emission at 681 nm (due to chlorphyll *a*) had reached a steady value and fluorescence emission spectra were then recorded. Then 20 mM NaCl and 250 μM inorganic carbon (as

$KHCO_3$) were added to the cell suspension and emission spectra were recorded after the fluorescence emission at 681 nm had reached its new lower value. Iodoacetamide (3.3 mM) and DCMU (20 μM) were added in subsequent additions. The chlorophyll concentration of the cell suspensions ranged from 3.9 to 4.2 μg/ml.

Results

Dark adapted *Synechococcus* cells, illuminated in the absence of inorganic carbon, showed a rapid rise in fluorescence followed by a slower increase to a maximum steady state value (Fig. 1A) as described by other authors [32–34]. The addition of 1 mM $KHCO_3$ to the cell suspension initiated a decrease in the fluorescence yield and a rapid rate of photosynthesis. During a subsequent period in darkness the cells were probed with 0.5 s flashes of the illuminating light and the fluorescence yields recorded (Fig. 1A and B). The yields declined slowly to a constant level, as described by Papageorgiou and Govindjee [35]. Upon continuous illumination, the fluorescence yield rose rapidly to a level equal to that elicited by the last 0.5 s flash and then rose slowly to a level equivalent to that in the previous light period (Fig. 1B). The addition of 15 μM DCMU at this time resulted in a rapid (less than 3 s) recovery of the fluorescence yield seen in the absence of photosynthesis (data not shown).

The results show that a 0.5 s flash given to dark-adapted cells served as a reasonable measure of the fast rise in fluorescence yield. These fast Kautsky transients [32–34] were not resolvable in our system, but since they amount to only about 10% of the total variable fluorescence yield [32–34] in cyanobacteria, it is not too important for our purposes to know what particular aspect of these very rapid processes were being measured (cf. Ref. 35).

On the basis of the results (Fig. 1) we will define variable fluorescence as $F_V = F_M - F_{0.5\ s}$, where F_M is the fluorescence yield in the absence of photosynthesis and $F_{0.5\ s}$ is the fluorescence yield from a 0.5 s flash of light given during a

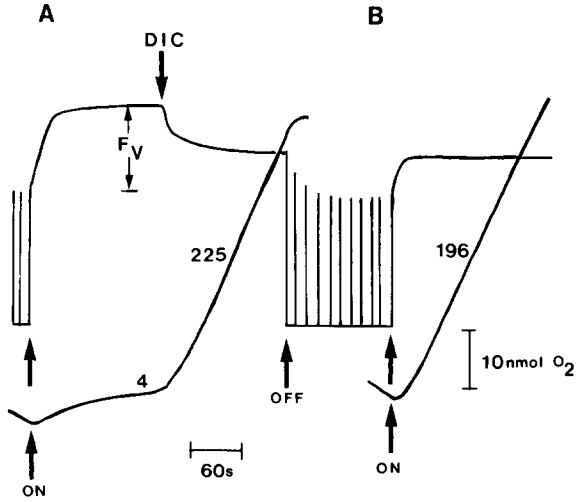

Fig. 1. The effect of inorganic carbon upon the slow fluorescence induction of dark-adapted cells. The cells were incubated in the light in the presence of 20 mM NaCl to remove any inorganic carbon from the solution and then incubated in the dark before the first illumination shown. In this, and subsequent figures, the upper trace represents the fluorescence yield and the lower trace represents O_2 evolution. Flashes (0.5 s) of light were used in the dark intervals between A and B. The photosynthetic rates are given as μmol O_2 per mg Chl per h. DIC, dissolved inorganic carbon (as 1 mM $KHCO_3$). F_v, variable fluorescence.

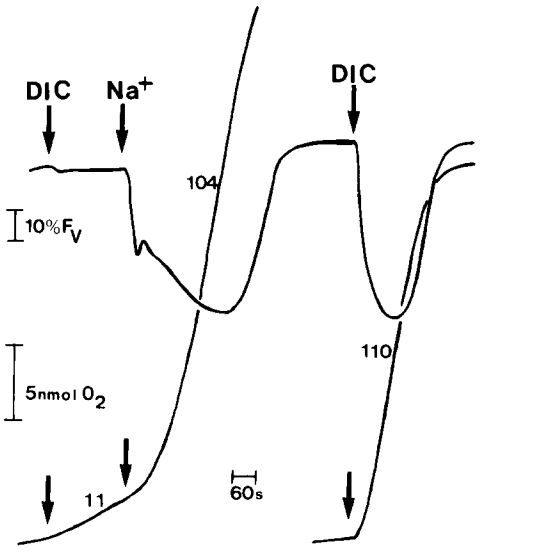

Fig. 2. The initiation of inorganic carbon transport by Na^+ and its effect upon fluorescence yield and O_2 evolution. The cells were under continuous illumination and transport was first initiated by the addition of 20 mM NaCl and subsequently by the addition of 25 μM dissolved inorganic carbon (DIC) (as $KHCO_3$). Photosynthetic rates are given as μmol O_2 per mg Chl per h.

dark period. In subsequent figures, fluorescence yield changes are described in terms of % F_V.

In the presence of Na^+, at pH 8.0 and above, the form of inorganic carbon that is transported by cells of *Synechococcus* UTEX 625 is mainly HCO_3^- [4,5,13]. In the absence of Na^+, little change in fluorescence was observed upon the addition of 25 μM $KHCO_3$ (Fig. 2), but upon the subsequent addition of Na^+ a large decrease in fluorescence occurred. The decrease in fluorescence yield was followed by a high rate of photosynthesis. The pattern could now be repeated by the addition of more dissolved inorganic carbon ($CO_2 + HCO_3^- + CO_3^{2-}$) (Fig. 2) and similar results were obtained at pH 9.6 (data not shown).

At pH 8.0, in the absence of Na^+, photosynthesis of *Synechococcus* cells is stimulated by the addition of carbonic anhydrase to the reaction medium [13]. Under these conditions, we assume that the cells are mainly using CO_2 as the form of inorganic carbon, and carbonic anhydrase has increased the availability of CO_2. The addition of dissolved inorganic carbon to cells in the absence of Na^+ resulted in a low rate of photosynthesis and a small amount of fluorescence quenching (Fig. 3). The following addition of carbonic anhydrase (Fig. 3) resulted in a dramatic decrease in fluorescence yield and a subsequent stimulation of O_2 evolution. When CO_2 availability was decreased by raising the pH of the cell suspension from 8.0 to 9.3 [13], the fluorescence yield increased and the subsequent addition of Na^+ then caused a reduction of the fluorescence yield (data not shown). The CO_2 concentration in solution can also be transiently raised above the equilibrium value by the addition of small volumes of CO_2 saturated water. At pH 8.0 most of the added CO_2 is eventually converted to HCO_3^-, but, in the absence of carbonic anhydrase, at a slow rate [8,9], which allows for CO_2 transport to occur in the first 10 s after addition [14]. In the absence of either Na^+ or carbonic anhydrase, the addition of small volumes of CO_2 saturated water caused a CO_2 concentration dependent lowering of the fluorescence yield (Fig. 4). The addition of CO_2 also caused a transient increase in the rate of O_2 evolution (data not shown). Note that very low concentration of inorganic carbon (1.7 μM) elicited observable changes in the fluorescence yield.

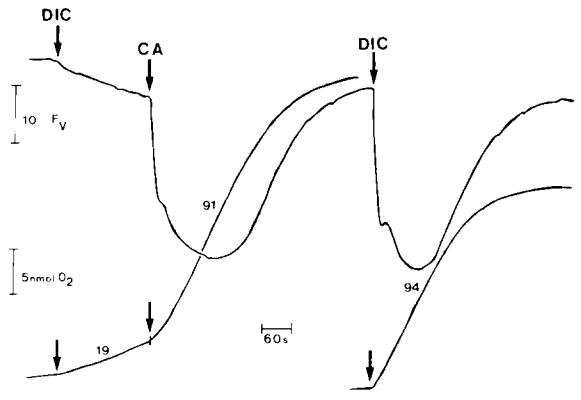

Fig. 3. The initiation of inorganic carbon transport by carbonic anhydrase and its effect upon fluorescence yield and O_2 evolution. The cells were under continuous illumination and transport was initiated by the addition of carbonic anhydrase (CA) (20 μg/ml) and subsequently by the addition of 25 μM dissolved inorganic carbon (DIC) (as $KHCO_3$). Photosynthetic rates are given as μmol O_2 per mg Chl per h.

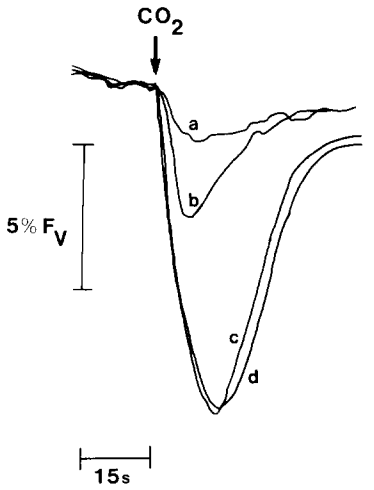

Fig. 4. Changes in fluorescence yield following the addition of CO_2. The cells were continuously illuminated in the absence of either NaCl or carbonic anhydrase. The CO_2 was added as small volumes of CO_2-saturated water. (a) 1.7 μM CO_2; (b) 3.5 μM CO_2; (c) 10.4 μM CO_2; (d) 17.3 μM CO_2.

The room-temperature fluorescence emission spectra for cells incubated in the presence and absence of inorganic carbon yields a difference spectrum with an emission peak at 680 nm (Fig. 5). This is consistent with changes in the fluorescence yield being due mainly to changes in the fluorescence of chlorophyll *a* in Photosystem II

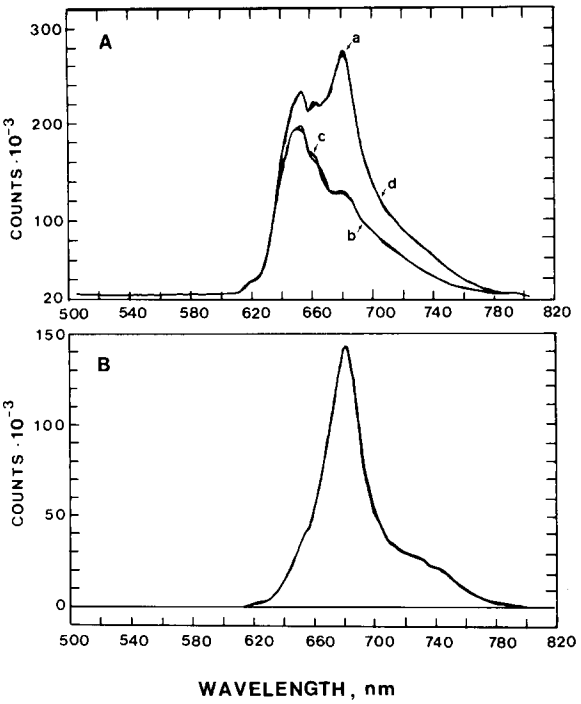

Fig. 5. (A) The fluorescence emission spectra at room temperature. The cells were incubated under continuous illumination and spectra were obtained under the following conditions: (a) no additions; (b) 20 mM NaCl and 250 μM $KHCO_3$; (c) as for (b), but with 3.3 mM iodoacetamide; (d) as for (c), but with 20 μM DCMU. (B) Difference spectrum of a − b.

[35,36] but some contribution above 700 nm from chlorophyll *a* in Photosystem I [33] can not be ruled out without low-temperature fluorescence measurements [32,34].

Iodoacetamide inhibits photosynthetic CO_2 fixation but allows normal transport and accumulation of inorganic carbon by *A. nidulans* [16]. Cells incubated with Na^+ and inorganic carbon yielded the same fluorescence emission spectrum whether or not 3.3 mM iodoacetamide was present (Fig. 5A). The addition of DCMU to iodoacetamide-poisoned cells restored the spectrum to that yielded by unpoisoned cells in the absence of Na^+ and inorganic carbon (Fig. 5A).

Only very low rates of net O_2 evolution were observed in *Synechococcus* cells inhibited by iodoacetamide (Fig. 6). But the reduction in fluorescence yield was similar to that in control cells upon the addition of HCO_3^- in the presence of Na^+ (Fig. 6A) or the addition of HCO_3^- in the

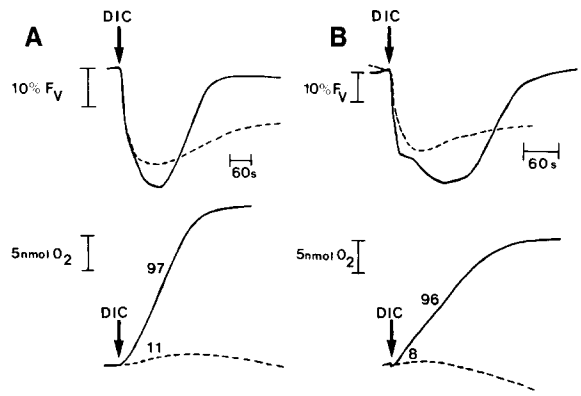

Fig. 6. Fluorescence yield and oxygen evolution from cells incubated without (solid line) or with (broken line) 3.3 mM iodoacetamide. (A) Cells incubated with continuous illumination in the presence of 20 mM NaCl. (B) Cells incubated with carbonic anhydrase (25 μg/ml). In both (A) and (B) transport was initiated by the addition of 25 μM dissolved inorganic carbon (DIC) (as $KHCO_3$). Photosynthetic rates are given as μmol O_2 per mg Chl per h.

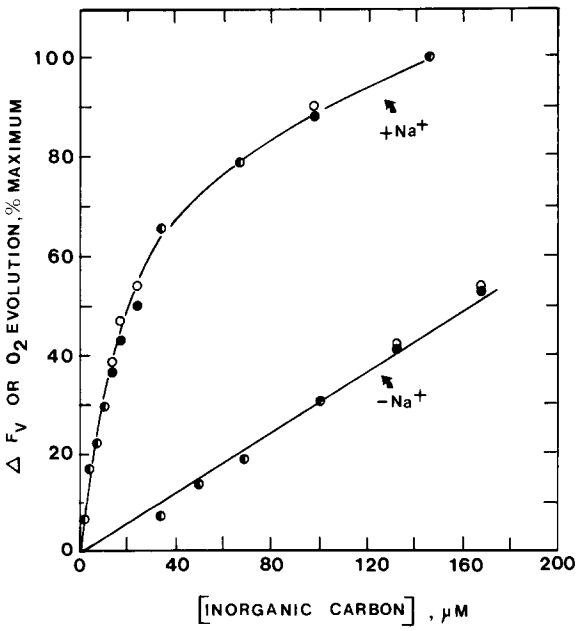

Fig. 7. The effect of inorganic carbon concentration upon the maximum extent of fluorescence quenching and the rate of O_2 evolution. Measurements were made either in the absence or presence of 20 mM NaCl. The fluorescence results are expressed as a % of the maximum change (59.8% F_v) in variable fluorescence that was observed. The O_2 evolution results are expressed as a % of the maximum rate (205 μmol per mg Chl per h) of O_2 evolution.

presence of carbonic anhydrase (Fig. 6B). Furthermore, it should be noted, because the carbon could not be fixed, that quenching of fluorescence continued for the period shown, although it declined slightly with some uptake of O_2. When CO_2 saturated water was added to cells in the presence of iodoacetamide, the CO_2 also caused fluorescence quenching but no net O_2 evolution occurred.

These results (Figs. 5 and 6) show that the observed fluorescence quenching was not due to CO_2 fixation by cyanobacteria, but rather was due to the transport and accumulation of inorganic carbon by the cells. Either HCO_3^- or CO_2 transport was equally effective.

For photosynthesis in the presence or absence of Na^+ (presumably sustained by CO_2) there was a very close correspondence between the maximum extent of fluorescence quenching at a given inorganic carbon concentration and the maximum rate of net O_2 evolution (Fig. 7).

Discussion

The reduction in fluorescence yield upon the initiation of photosynthetic CO_2 fixation has been observed previously in algae, chloroplasts and leaves [17,19,20–28,33,37] and a close relationship between the extent of fluorescence quenching and the rate of photosynthesis has recently been shown for spinach leaves [22]. We show (Fig. 7) that in cyanobacteria there was also a close correspondence between the extent of fluorescence quenching and the rate of net O_2 evolution over a wide range of external carbon concentration. At first glance this would suggest that the fluorescence quenching was due to CO_2 fixation but, the results of Figs. 5 and 6 clearly show that this is not so because the same quenching was observed in the absence of CO_2 fixation. The explanation for the close correspondence must lie elsewhere. The rate of net photosynthesis in the cyanobacteria is completely dependent on the size of the internal pool of inorganic carbon [2,7,13]. The size of this internal pool, in turn, is dependent on the external concentration of inorganic carbon and the rate of transport of inorganic carbon into the cells [2,7]. As fluorescence quenching is not due to CO_2 fixation it must be due either to the transport or the magnitude of the internal pool of inorganic carbon. If the extent of fluorescence quenching was due either to the rate of transport or the magnitude of the internal pool it was not surprising that the maximum extent of quenching would correspond to the maximum net O_2 evolution rate, since the latter was dependent on the same features. It is important to note that there is always a time difference between the quenching of fluorescence and initiation of high rates of photosynthesis (Figs. 2, 3 and 6). But maximum quenching and maximum photosynthesis occur at the same time. This may argue that quenching is due to the magnitude of the internal pool but, at the moment, we do not know whether the quenching resulted from the direct energy demand of transport or from the accumulation of large amounts of inorganic carbon within the cells. Inorganic carbon concentrations in cyanobacteria can be 20 mM or higher [1,2,4,7,16,13] and the effect of these high concentrations upon the photosynthetic electron transport chain is not known.

Changes in fluorescence yield in chloroplasts and leaves induced by CO_2 are attributed to either Q-quenching (due to the oxidation of Q) or E-quenching (due to the transthylakoidal pH gradient) [18,21,23–25,28]. Although some slow fluorescence changes occur in *A. nidulans* that correlate with structural changes in the thylakoids [39], E-quenching has not been established for cyanobacteria. The rapid relief of quenching by the addition of DCMU [38] (Fig. 5) suggests that the quenching observed is Q-quenching. The occurrence of quenching, however, when CO_2 fixation is inhibited (Fig. 6) seems to contradict this view. One should recognize, however, that only net O_2 evolution (which would result in CO_2 fixation) was measured. If zero net O_2 evolution is due to the equal uptake and evolution of oxygen upon the initiation of inorganic carbon transport, Q could still be oxidized by a pathway to O_2 that has not yet been described.

Acknowledgements

This study was supported by a grant from the Natural Sciences and Engineering Research Council of Canada to D.T.C. We also thank Dr. R.H. Pottier and Mr. Glen Weagle of the Department of Chemistry and Chemical Engineering,

Royal Military College of Canada, Kingston, Ontario, Canada for their enthusiastic help in the measurement of the fluorescence emission spectra. Dr. George Espie provided both technical assistance and valuable advice.

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