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Antagonistic effects of light I and II on chlorophyll *a* fluorescence yield and P700 turnover as monitors of carbon dioxide depletion in intact algal and cyanobacterial cells

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Depletion of carbon dioxide from cells by formate treatment not only causes a cessation of carbon dioxide fixation, but also a dramatic decrease in the rate of electron transfer between Q_A, the primary plastoquinone electron acceptor of photosystem II, and the cytochrome b_6/f complex. We show here that this latter phenomenon can be conveniently monitored by the antagonistic effects of light absorbed in photosystems I and II on chlorophyll *a* fluorescence yield and P700 turnover in intact cells of green algae and cyanobacteria.

Key words – Algae, bicarbonate effect, cyanobacteria, electron transport, fluorescence, light-state transitions, photosynthesis, P700.

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Introduction

Depletion of carbon dioxide from thylakoids, by formate or NO treatment, causes a strong inhibition of photosynthetic electron transport. The thylakoids can be reactivated by a short dark-incubation with bicarbonate. This effect is named the bicarbonate-effect (see reviews by van Rensen and Snel 1985, Blubaugh and Govindjee 1988, van Rensen 1993, Govindjee and van Rensen 1993). Most of the research on the bicarbonate-effect has been performed with isolated chloroplasts. There are relatively few reports about the bicarbonate-effect in intact organisms (Garab et al. 1983, Mende and Wiessner 1985, Ireland et al. 1987, Garab et al. 1988, Cao and Govindjee 1988, El-Shintinawy and Govindjee 1990, El-Shintinawy et al. 1990, Govindjee et al. 1991).

The observation of a bicarbonate-effect on electron transport between the two photosystems in vivo is difficult to distinguish from that due to the CO_2 fixation activities in the Calvin cycle. Ireland et al. (1987) studied the bicarbonate-effect in maize leaves by lowering the CO_2 to a level where CO_2-fixation activity was insignificant and small increases in CO_2 concentration led to reoxidation of Q_A^- without changes in CO_2 fixation. Canvin and coworkers (Miller et al. 1988, Miller and Canvin 1989) observed fluorescence changes due to Q_A^- oxidation upon the addition of bicarbonate to *Synechococcus* UTEX 625 cells in which CO_2 fixation was inhibited with iodoacetamide or glycoaldehyde. In their experiments, O_2 was suggested to serve as an electron acceptor facilitating electron transport coupled with photophosphorylation; the ATP being used for inorganic carbon uptake. Cao and Govindjee (1988) inhibited electron flow beyond plastoquinol by DBMIB. After adding bicarbonate to CO_2-depleted/formate-treated intact *Synechocystis* cells, they observed reactivation of photosystem II dependent electron flow.

Siggel et al. (1977) had shown that CO_2-depletion of

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spinach thylakoids by formate treatment leads to a slowed rereduction of oxidized P700 (the reaction center chlorophyll *a* of photosystem I). Govindjee et al. (1960) and Duysens and Sweers (1963) had already shown the potential of using light I and light II (light absorbed by PSI and PSII, respectively) on chlorophyll *a* fluorescence yield in studying electron flow in photosynthesis.

In this paper, we have studied the bicarbonate-effect in intact cells of the green alga *Scenedesmus obtusiusculus* and in those of the cyanobacterium *Synechocystis* PCC 6803 by measuring chlorophyll *a* fluorescence yield and P700 turnover after successive exposure to light absorbed in PSI and PSII. These processes are not directly influenced by Calvin cycle activity and, thus, have allowed us to monitor CO_2-depletion effects without the use of inhibitors other than formate.

Abbreviations – Chl, chlorophyll; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; λ_I, light absorbed by PSI; λ_{II}, light absorbed by PSII; P700, reaction center chlorophyll *a* of PSI.

Materials and methods

Plant material

Green unicellular algae (*Scenedesmus obtusiusculus* Chod.) were cultivated as described earlier (van Rensen 1975). The cyanobacterium (*Synechocystis* sp. PCC 6803) was grown in BG11 medium (Rippka et al. 1979) in the same setup with additional light (44 μmol m^{-2} s^{-1}) from a 25 W tungsten lamp.

CO_2 depletion/formate treatment

The cells were depleted of CO_2 as described earlier for isolated chloroplasts (Snel and van Rensen 1984). Algae (40 ml, 5 μl cells ml^{-1}, packed cell volume) were centrifuged, washed and suspended in water. Then, the cells were washed once with 4 ml CO_2-depletion medium (0.3 *M* sorbitol, 50 m*M* sodium formate, 5 m*M* $MgCl_2$, 10 m*M* NaCl, 25 m*M* MES pH 5.8), suspended in 4 ml CO_2-depletion medium and the depletion procedure was started. The reaction medium was the same, except that formate was omitted and the pH was 6.5. Measurements were carried out at 25°C and all media were depleted of CO_2 by means of bubbling with CO_2-free nitrogen (99.99%) gas.

In some experiments, cells were depleted of CO_2 without the use of any inhibitor; they were first allowed to photosynthesize in a closed chamber in saturating red light when *S. obtusiusculus* was used and in saturating yellow-orange light when *Synechocystis* PCC 6803 was used. This method that often lasted one or two h lowered the CO_2 content to a very low level such that the rates of photosynthesis diminished to less than 5% of the control in saturating light.

Chlorophyll *a* fluorescence

Chlorophyll *a* fluorescence transients were measured using a PAM fluorometer (Walz, Effeltrich, Germany) connected to one optical port of a thermostatted cuvette (DW2/2, Hansatech Instruments Ltd., Kings Lynn, Norfolk, UK). Light I (λ_I) was generated by a home-built tungsten-halogen light source, passed through an optical filter (RG 695) and focussed onto one arm of the 101-F fibre-optic light guide of the PAM fluorometer. Light II (λ_{II}) was provided by a tungsten-halogen source (LS2, Hansatech Instruments Ltd.), filtered through 640 nm filter (see legends to Figs 1 and 2 for details) and led to the cuvette by fibre-optics (type A8, Hansatech Instruments Ltd.). The baseline fluorescence yield in λ_{II} light was adjusted to start at the same position on the chart paper in the different samples for ease in comparison of changes induced by λ_I light. For both CO_2-depleted and NaF-treated samples, the baselines were brought down to the control or the CO_2-reactivated sample baseline. All measurements were, however, made on the same scale.

Absorption changes at 820 nm

P700 transients were measured at 820 nm using the PAM fluorometer equipped with the ED-800 emitter-detector head (Walz, Effeltrich, Germany). Light I (λ_I) was obtained as described above using a Balzers 720 nm (10 mn band width) interference filter (B221). Light II (λ_{II}) was obtained from a second home-built tungsten-halogen source equipped with an electro-mechanical shutter, and led to the cuvette via optical filters and the remaining arm of the 101-F fibre optics of the PAM fluorometer. Either a combination of Schott BG 38 + OG 570 filters (λ_{peak} = 578 nm, band width 50 nm; Fig. 3) or Baird Atomic (BA 38) interference + Balzers Calflex C filters (λ_{peak} = 650 nm, band width 10 nm) (Fig. 4) were used for light II.

Irradiance, in W m^{-2}, was measured with an optometer (UDT model 370) at the end of the optical fibre and are indicated in the legends of the figures.

Results and discussion

Chlorophyll *a* fluorescence

Excitation of cells with light absorbed mainly by photosystem II (λ_{II}: 640 ± 10 nm) led to an increased Chl *a* fluorescence yield (not shown) as has been well documented (see e.g. Duysens and Sweers 1963). Experiments reported in Fig. 1 were done with cells preilluminated with λ_{II} for 10 min. The primary plastoquinone acceptor Q_A is then largely in the reduced state, but since the dark-adapted cells are in the so-called State II

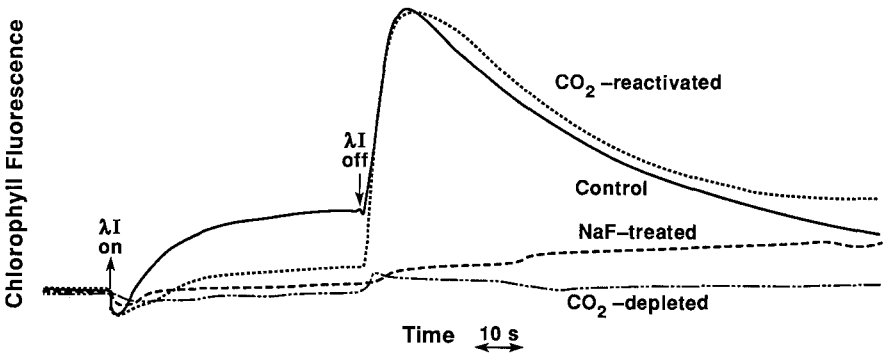

Fig. 1. Chlorophyll *a* fluorescence yield changes (in arbitrary units, but on the same scale) in *Synechocystis* sp. PCC 6803 cells preilluminated for 10 min with λ_{II} (640 nm). See text for details on the measurement protocol. Experimental conditions: λ_{II}: 640 nm, 50 W m^{-2}; λ_I: RG 695 filter, 100 W m^{-2}; 12.5 µl cells ml^{-1}. Data on control, CO_2-depleted, CO_2-reactivated, and NaF-treated samples are shown. The baseline fluorescence yields before λ_I was given are adjusted to the same level.

(see e.g. Fork and Satoh 1983, Allen et al. 1989) the Chl *a* fluorescence yield is not maximal. Additional illumination with λ_I (RG 695 filter) led to a transient quenching of cells (see Govindjee et al. 1960, Duysens and Sweers 1963; see Fig. 1 for our data on *Synechocystis* 6803), but the Chl *a* fluorescence yield increased as the cells went from State II to State I. As soon as λ_I was turned off, there was an immediate increase in Chl *a* fluorescence yield as now all Q_A could be in the Q_A^- state. This was followed by a transition to State II and the Chl *a* fluorescence yield declined.

Depletion of CO_2 from cells by photosynthesis (about 1 h) led to a drastic elimination of the state changes that are dependent upon the changing concentration of plastoquinol and a dramatic decrease in the amplitude of fluorescence change due to Q_A^- component after λ_I is turned off (data not shown). Restoration of the CO_2 level by dark respiration led to 60–80% recovery of the phenomenon. Since we found that simple addition of CO_2 did not restore the phenomenon immediately, it became obvious that we were dealing here with the superimposition of metabolic regulation of state changes similar to that reported by Turpin and Bruce (1990). Because it was difficult to separate the "bicarbonate-effect" from the metabolic effect, we performed the experiments with bicarbonate-depleted (formate-treated) *Synechocystis* 6803 cells.

In formate-treated/bicarbonate-depleted cells (labeled as CO_2-depleted), we observed that both the quenching of Chl *a* fluorescence by λ_I as well as the state changes that depend upon the plastoquinone pool were eliminated or drastically reduced. However, addition of bicarbonate to such cells (CO_2-reactivated) restored the Chl *a* fluorescence yield changes to essentially that of the control values (Fig. 1). The absence of state changes in CO_2-depleted cells, observed here, is reminiscent of similar effects in cyt b_6/f lacking mutants of *C. reinhardtii* (Wollman and Lemaire 1988). Thus, a block in the intersystem chain leads to similar results. Furthermore, NaF abolished the state changes (Fig. 1) in agreement with state changes being responsible for the large fluorescence changes (see a review by Allen 1992).

An interesting observation by Allen et al. (1989) was that state changes were qualitatively different in *Synechococcus* 6301 cells grown at high-intensity λ_I and those grown at λ_{II}. We observed during our work that only 10 min preillumination with λ_I and λ_{II} can produce similar differences in our *Synechocystis* 6803 cells. Figure 2 shows results with cells pre-illuminated for 10 min with λ_I, followed by brief (20 s) illumination with λ_{II}.

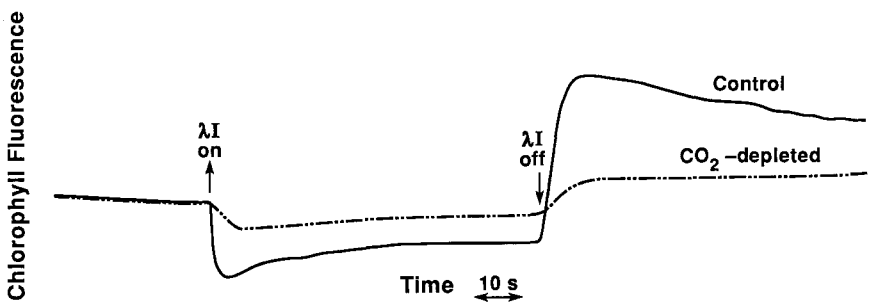

Fig. 2. Chlorophyll *a* fluorescence yield changes (in arbitrary units, but on the same scale) in *Synechocystis* sp. PCC 6803 cells preilluminated for 10 min with λ_I (RG 695), followed by 20 s illumination with λ_{II}. See text for details on the measurement protocol. Experimental conditions are the same as in Fig. 1. Control and CO_2-reactivated sample were identical and are superimposed on each other. The baseline fluorescence yields before λ_I was given are adjusted to the same level.

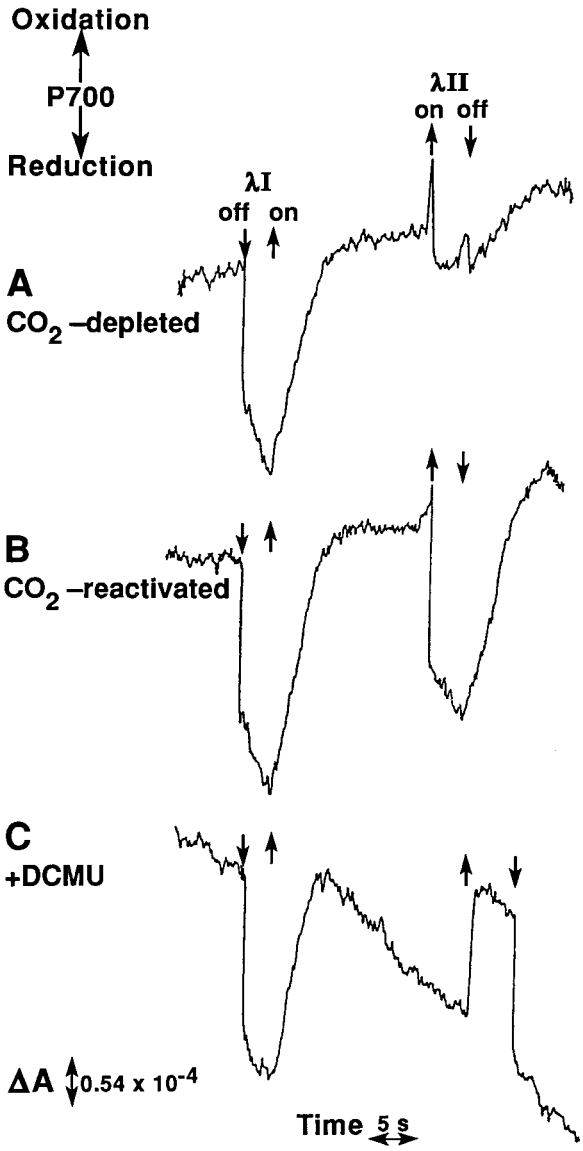

Fig. 3. Light-induced apparent absorption changes at 820 nm in a suspension of *Synechocystis* sp. PCC 6803 cells. See text for details on the measurement protocol. Experimental conditions: λ_I: 720 nm, 48 W m^{-2}; λ_{II}: 578 nm, 46.5 W m^{-2}; 25 μg Chl ml^{-1}. The integrated intensity of the 820 nm measuring light was 0.4 mW cm^{-2}.

The state changes in this case were of different magnitude than in cells preilluminated for longer time (10 min) with λ_{II} (Fig. 1). However, the effects of CO_2-depletion by photosynthesis and by formate treatment were quantitatively similar in the two cases. CO_2-depletion decreased significantly the quenching of Chl *a* fluorescence by λ_I, and this quenching as well as state changes are recovered by bicarbonate addition. Results with CO_2-reactivated and control samples were superimposed on each other. DCMU-treated samples showed results (data not shown) similar to those published by Duysens and Sweers (1963).

ΔA820 measurements

Algae were adapted to λ_I in the cuvette until a constant baseline was obtained with P700 in a partially oxidized state. In order to circumvent noise induced by the stirrer and the flow of water through the water mantle of the cuvette and reduce large apparent absorption changes induced by λ_{II} (J. F. H. Snel, unpublished observations), the magnetic stirrer and the pump of the thermostat were stopped. As soon as the baseline was constant again, λ_I was switched off for 5 s to reduce P700. Then, after about 20 s relaxation to a steady state in λ_I, λ_{II} was added to λ_I for 5 s to see whether PSII was able to reduce the oxidized P700 ($P700^+$).

Figure 3 shows typical ΔA820 transients measuring P700, obtained using the protocol described above, from CO_2-depleted (formate-treated, A), CO_2-reactivated (10 m*M* $NaHCO_3$, B) and CO_2-reactivated but DCMU-treated (10 μ*M* DCMU, C) *Synechocystis* sp. 6803 cells. Here λ_{I+II} was obtained by adding to light I (B221 filter, 720 nm), light from Schott BG 38 + OG 570 filters (578 ± 50 nm). Since reversibility by bicarbonate is important in establishing the "bicarbonate effect", and since control samples gave results similar to CO_2-reactivated samples, only the latter are shown. Furthermore, (1) experiments with NaF-treated samples were considered unnecessary to establish the effect of CO_2-depletion on the redox state of P700, an important focus of this paper; but (2) experiments with DCMU-treated samples were considered essential as an additional control for showing how a total blockage between the two systems leads to changes in the redox level of P700. Trace A shows that in the CO_2-depleted/formate-treated cells illumination of the cells with λ_{I+II} leads only to a small transient absorption change (reduction of P700), but not to a significant decrease in [$P700^+$] as judged from the difference between the amplitude with λ_I off and on. This result is consistent with the inhibition of electron flow between PSII and PSI in formate-containing media (in the absence of CO_2). Addition of bicarbonate (trace B) does not affect the amplitude of the absorbance change difference between the turning off and on of λ_I, indicating that electron flow in PSI is not influenced by bicarbonate. However, the addition of λ_{II} induces a large apparent absorption decrease (reduction of [$P700^+$] by PSII). The amplitude of this absorption change indicates that the P700 is reduced to about the same level as in the dark. As a control, 20 μ*M* DCMU was added to the CO_2-reactivated cells (trace C). Although there is more drift in the baseline in this experiment , the effect of turning λ_I off and on is similar to that in the the CO_2-reactivated sample. However, in the presence of DCMU, λ_{II} induces an opposite absorption change, suggesting that P700 is even further oxidized by λ_{II} due to total blockage

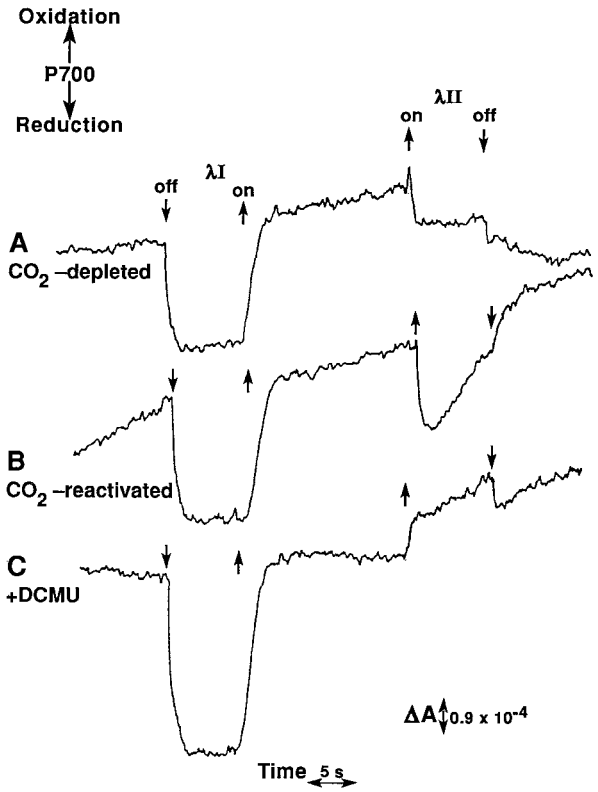

Fig. 4. Light-induced apparent absorption changes at 820 nm in a suspension of *Scenedesmus obtusiusculus* cells. See text for details on the experimental protocol. Experimental conditions: λ_I: as in Fig. 3, 25 W m^{-2}; λ_{II}: 650 nm, 170 W m^{-2}; 25 μg Chl ml^{-1}. The integrated intensity of the 820 nm measuring light was 0. 4 mW cm^{-2}.

of electron flow from PSII to PSI: even light II, that must 'spillover' to PSI, causes oxidation of P700.

A similar experiment carried out with *S. obtusiusculus* is shown in Fig. 4. These results resemble those obtained with *Synechocystis* sp. 6803. CO_2-depletion/formate-treatment abolishes or decreases the reduction of oxidized P700, whereas bicarbonate addition allows the reduction of P700 (see the effect of λ_{II}). An obvious difference, however, between the two species is the amplitude of the response to λ_{II} in the presence of DCMU. This reflects the differences in the concentration of the reduced P700 in the dark among the two species (compare Fig. 3C with 4C).

We conclude that CO_2-depletion/formate-treatment of both the cyanobacterium *Synechocystis* sp. PCC 6803 and the green alga *S. obtusiusculus* induces a state in which electrons from PSII cannot reduce oxidized P700 and that dark-incubation with bicarbonate (or CO_2) reverses this effect. Thus, both the antagonistic effect of λ_I and λ_{II} on Chl *a* fluorescence yield changes and the redox state of P700 become useful tools to monitor the bicarbonate-effect.

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