Reversible inhibition of photochemistry of photosystem II by Ca^{2+} removal from intact cells of *Anacystis nidulans*

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Depletion of Ca^{2+} from *Anacystis nidulans* produces an inhibition of O_2 evolution that is accompanied both at 39°C and 77 K by a loss of chlorophyll fluorescence of variable yield. This indicates that Ca^{2+}-depletion causes disruption of normal photosystem II function, manifested by the disappearance of photoreduction of Q. Delayed light emission in the ms time range is also eliminated in Ca^{2+}-depleted cells, which confirms that Ca^{2+} removal prevents charge separation and recombination in reaction centers of photosystem II. Readdition of Ca^{2+} to depleted cells restores fully the fluorescence of variable yield and delayed light emission, as well as O_2 evolution. Thus, Ca^{2+} may be a required component for photosystem II in *A. nidulans*.

Anacystis nidulans *Blue-green algal photosynthesis* *Calcium inhibition* *Cyanobacteria* *Photosystem II* *Variable fluorescence*

1. INTRODUCTION

Membrane preparations from *Anacystis nidulans* [1,2] and other [3,4] blue-green algae (cyanobacteria) require Ca^{2+} for optimum photosystem II (PS II) activity. Actively growing cells of *A. nidulans* lose PS II activity when Ca^{2+} is removed from the growth medium, while photosystem I (PS I) is unaffected [5]. Since addition of Ca^{2+} to the growth medium restores O_2 evolution, it appears that this ion is necessary for the proper functioning of PS II. To investigate in detail how Ca^{2+} affects the photochemical activity of PS II, we measured kinetics of the reduction of *Q*, the primary stable acceptor of PS II [6]. For these studies we measured both the kinetics of fluorescence and the magnitude of ms-delayed fluorescence. The results demonstrate that Ca^{2+} depletion arrests the very early photoreactions of PS II.

2. MATERIALS AND METHODS

Anacystis nidulans TX 20 (UTEX 625, Collection of Algae, University of Texas, Austin, TX 78712) was maintained at 39°C in late log growth. A continuous culture chamber was aerated with 3% CO_2 under constant illumination as in [5]. For depletion experiments cells were transferred to Cg-10 medium [7] modified to contain 1.5-times the normal amount of glycylglycine buffer without $Ca(NO_3)_2$ [5]. Cells from the continuous culture chamber were washed once by centrifugation through this medium then resuspended in the same medium to a chlorophyll [chl] concentration of about 5 $\mu g.ml^{-1}$. $Ca(NO_3)_2$ was added to a final concentration of 0.35 mM in control preparations. Control cells or cells lacking Ca^{2+} were aerated at 39°C with 3% CO_2 and illuminated with

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300 $\mu E.m^{-2}.s^{-1}$ of red light ($\lambda > 600$ nm) obtained by filtering light from 350 W incandescent lamps through red plastic (Rohm and Haas no.2423). Cells were aerated in darkness at 39°C with 5% CO_2 for at least 10 min prior to measurements of O_2 evolution or fluorescence.

Oxygen evolution was measured in undiluted cultures using a Clark-type electrode (Rank, Cambridge), illuminated with saturating red light (Schott RG2 and Balzers Calflex C heat-reflecting filter). [Chl] was determined by extracting cells in 80% acetone, measuring the absorbance at 663 nm, and applying an extinction coefficient of 82.04 $ml.mg^{-1}$ [8].

Time courses of chl *a* fluorescence at 685 nm were measured using a fiber optic system to excite and collect the fluorescence from the upper surface of the sample [9]. Blue excitation light (λ = 442 nm) was provided by a HeCd laser (Liconix, model 4240) attenuated with appropriate neutral density filters. Blue-green light was obtained by passing the light from a 150 W, 21.5 V projector lamp (type DLS) through two Corning glass filters (CS 4-96) and a Calflex-C heat-reflecting filter (Balzers). For measurements of fluorescence induction at 39°C in the ms time range a fast oscillographic recorder was used (Massa Meterite, model 301). Fluorescence induction at 77 K could be measured with a standard strip chart recorder if the intensity of the actinic light was kept low enough.

Delayed fluorescence in the ms time range was measured with a phosphoroscope, the rotating sector (EG&G, PARC Mod. 125A) of which provided alternating light/dark cycles of 0.8 and 5.1 ms, respectively. The resulting signal was amplified by a lock-in amplifier (EG&G PARC Mod. 128A) tuned to the chopper.

3. RESULTS

Fig.1 shows the time course of fluorescence in the presence of DCMU (3-[3,4-dichlorophenyl]1,1-dimethylurea) for control cells and cells that were depleted of Ca^{2+} so that they no longer evolved O_2. Control cells (——) showed an increase of variable fluorescence yield (F_v) upon illumination, as the stable primary PS II acceptor (Q) went from the oxidized to reduced state [6]. By contrast, cells lacking Ca^{2+} (– – –) had no F_v and had an F_o level

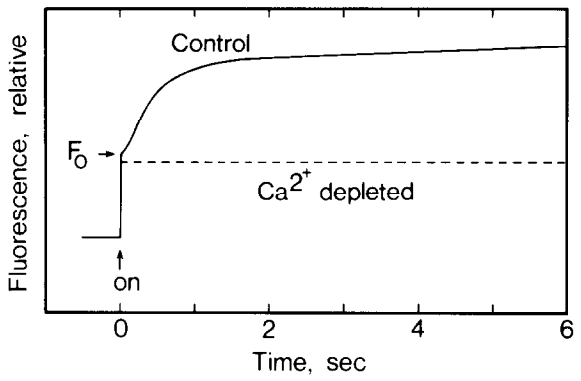

Fig.1. Time course at 39°C of chl fluorescence at 685 nm in *A. nidulans* illuminated with blue light (442 nm) in the presence of 20 μM DCMU. Cells were incubated for 8 h in red light in the presence (——) or absence (– – –) of Ca^{2+}. Curves were normalized to correct small differences in [chl] between control and Ca^{2+}-depleted cells. The arrow indicates the time when illumination began.

about the same as that of the control. The addition to control cells of high concentration (10 mM) of NH_2OH, which donates electrons to PS II [10], produced a more rapid fluorescence rise to maximal level than in control cells, followed by a decrease to a steady state level within 45 s (fig.2). In Ca^{2+}-depleted cells the fluorescence remained near the F_o level, and was not altered upon the addition of NH_2OH (fig.2). This suggests that Ca^{2+} depletion does not inhibit PS II by affecting the O_2-evolving site.

There was a proportionate loss of both O_2 evolution and fluorescence of variable yield (measured in the presence of DCMU) in cells partially depleted of Ca^{2+} (table 1). The time courses of the rise in variable fluorescence were essentially identical in control and in partially depleted cells, with half-times remaining at about 0.4 s (not shown). This indicates that the efficiency of energy transfer from light-harvesting chl to PS II reaction centers is not diminished by Ca^{2+} depletion, since such an effect would slow the time course of the fluorescence rise. Thus, loss of O_2 evolution appears to result directly from diminished photochemistry in Ca^{2+}-depleted cells.

Since control cells of *A. nidulans* treated with DCMU show a slow fluorescence rise that is associated with state II–I transitions [10,11] and since Ca^{2+} depletion also affects this slow

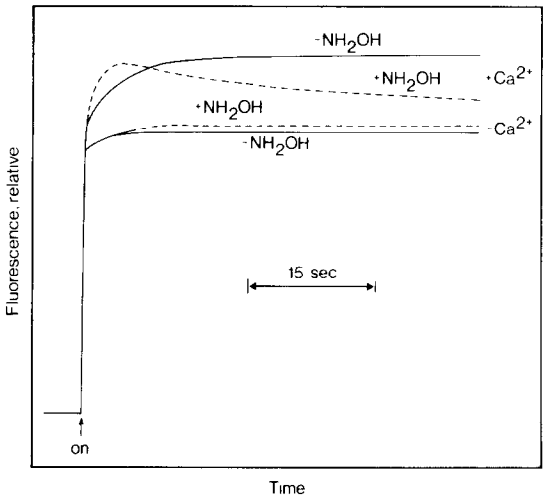

Fig.2. Time course of chl fluorescence in presence or absence of NH_2OH. Control and Ca^{2+}-depleted cells (20% of control O_2 evolution) were illuminated and fluorescence measured as described in fig.1. NH_2OH (10 mM) was added directly to the sample and equilibrated in complete darkness prior to fluorescence measurements: (——) no addition; (···) NH_2OH addition.

fluorescence rise, we measured time courses of fluorescence in dark-adapted cells at 77 K. State transitions do not occur at low temperatures [12], while the photochemical reduction of Q can still take place. The measurements at 77 K (fig.3) demonstrated variable fluorescence in control cells (——), but not in cells depleted of Ca^{2+} (– – –). The variable fluorescence measured at 77 K diminished with time in parallel with the decrease in O_2 evolution (table 2).

Table 1

Progressive loss of O_2 evolution activity and variable fluorescence yield in control and in Ca^{2+}-depleted cells

Time (h)	O_2 evolution		F_v (% control)
	Rate	% control	
0	322	100	100
3	161	50	56
5	56	17	12
7	0	0	0

Calcium depletion and fluorescence measurements were done as described in fig.1. [Chl] was 3 $\mu g \cdot ml^{-1}$. Rates of O_2 evolution expressed in $\mu mol\ O_2 \cdot mg\ chl^{-1} \cdot h^{-1}$

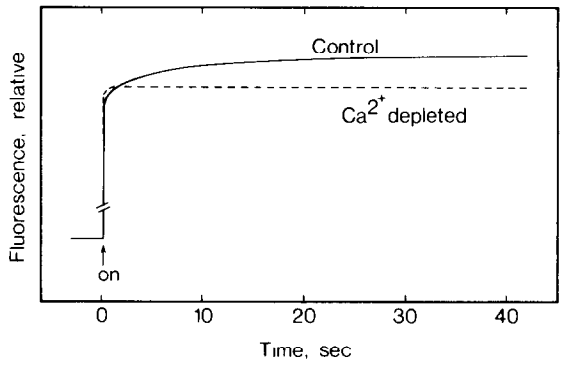

Fig.3. Time course at 77 K of chl fluorescence at 685 nm in *A. nidulans*. Cells were incubated in red light for 8 h in the presence (——) or absence (– – –) of Ca^{2+} during which time the O_2-evolving capacity of the minus-Ca^{2+} cells decreased to zero. Fluorescence was measured in undiluted cells in growth medium with no other addition. Fluorescence was excited by blue-green light ($30\ \mu E \cdot m^{-2} \cdot s^{-1}$) as described in section 2. Samples were kept in the dark for 10 min prior to cooling to 77 K for the fluorescence measurements.

The results shown in fig.3 and table 2 were obtained using a broad band of blue-green light that excited both phycocyanin and chl *a*. Identical results were obtained with monochromatic blue light (442 nm) that excited chl *a* almost exclusively. These results suggest that loss of fluorescence of variable yield was not caused by a loss of energy transfer from phycocyanin to chl *a*, but by a loss in the capacity of PS II reaction centers to reduce Q.

Table 2

Progressive loss of O_2 evolution activity and variable fluorescence (77 K) in control and in Ca^{2+}-depleted cells

Time (h)	O_2 evolution		F_v (% control)
	Rate	% control	
4	408	100	100
5	183	45	56
6	127	31	38
7	70	17	38
8	0	0	0

Calcium depletion and fluorescence experiments were done as in fig.2. [Chl] was 5 $\mu g \cdot ml^{-1}$. Rates of O_2 evolution given in $\mu mol\ O_2 \cdot mg\ chl^{-1} \cdot h^{-1}$

A comparison of tables 1 and 2 illustrates that the time required for Ca^{2+} depletion was dependent upon the light intensity to which cells were exposed in Ca^{2+}-deficient medium. Low incident light intensity (not shown) or high cell density (table 2 *vs* table 1) extended the lag time before symptoms of Ca^{2+} depletion were observed. However, the time courses of loss in O_2 evolution and fluorescence of variable yield were always parallel.

Since ms-delayed light emission is associated with charge recombination in PS II [13], we compared the delayed fluorescence in control cells and cells depleted of Ca^{2+}. The delayed fluorescence in control cells was similar to that reported for *A. nidulans* [14], while no delayed fluorescence was detected in Ca^{2+}-depleted cells. The magnitude of the delayed fluorescence signal diminished in parallel with O_2-evolving capacity during Ca^{2+} depletion of the cells (table 3).

To investigate whether Ca^{2+} depletion might change fluorescence yield by accelerating Q reoxidation, we measured in the presence of DCMU the time course of the dark reoxidation of reduced Q in control cells and in cells partially depleted of Ca^{2+}. Time courses of reoxidation of Q were about the same for both control cells (——) and for cells in which Ca^{2+} depletion had diminished O_2 evolution by 45% (fig.4). The half-time for reoxidation was about 0.5 s for both. Cells totally depleted of Ca^{2+} (···) showed virtually no variable fluorescence, precluding the possibility of measuring reoxidation of Q.

Table 3

Effect of Ca^{2+} depletion on delayed fluorescence emission in *A. nidulans*

	O_2 evolution		F_v (%
	Rate	% control	control)
Control	504	100	100
Partially depleted	184	37	48
Totally depleted	0	0	0

The ms delayed fluorescence was measured at 663 nm using a HeNe laser (Spectra-Physics Model 124B) as described in section 2. Height of the transient peak was measured and expressed as percentage of the height in control cells of the same [chl]. Rates of O_2 evolution given in μmol O_2.mg chl^{-1}.h^{-1}

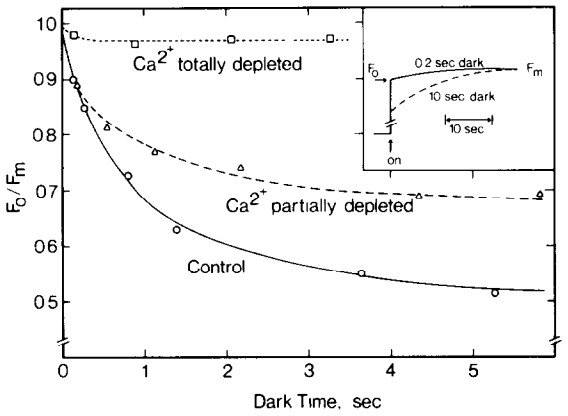

Fig.4. Kinetics of dark restoration of fluorescence of variable yield (685 nm) at 39°C in control cells (——) and in cells partially (– – –) or totally (···) depleted of Ca^{2+}. The samples were illuminated for 30 s with 442 nm light (1% of full intensity), given dark periods of varying times, and illuminated again with the 442 nm light to obtain F_o- and F_m-values measured as shown in the insert. Partially depleted cells (– – –) retained 45%, while totally depleted cells (···) retained less than 5% of the control O_2 evolution capacity.

4. DISCUSSION

Only a small amount of chl *a* of blue-green algae is associated with PS II [15], and light absorbed by phycobilins is transferred with high efficiency to chl *a* of PS II [15]. Loss of variable fluorescence could arise as a result of a decrease in energy transfer from phycobilins to chl *a*. Therefore, in most of these experiments we used monochromatic blue light (442 nm), absorbed almost exclusively by chl *a*. Calcium depletion caused a loss of variable fluorescence yield when this blue actinic light was used (fig.1, table 1). Thus, the loss of variable fluorescence did not result from a disruption of energy flow from phycobilins to chl *a*. The time course of the fluorescence rise in blue light was not altered in partially depleted cells, although the magnitude of the rise was less. This suggests that energy transfer from bulk chl *a* to reaction centers was not impaired. Ca^{2+} depletion must, therefore, affect either photochemical events or secondary electron transport associated with PS II. Since Ca^{2+} depletion inhibited variable fluorescence at 77 K (fig.3) and eliminated delayed fluorescence, the effect must be near the reaction

center of PS II. This is supported by the result shown in fig.2 where a high concentration of NH_2OH was unable to restore the variable fluorescence in Ca^{2+}-depleted cells. NH_2OH is known to feed electrons to PS II near the reaction center [10,13].

Ca^{2+} depletion diminished variable fluorescence without affecting the constant (F_o) component (fig.1). The specific loss of only the fluorescence of variable yield suggests that Ca^{2+} depletion leads to an accumulation of $P680^+$ [16], an idea that has been proposed for UV-inhibited [17,18] and ferricyanide-treated [19] chloroplasts, and for photoinhibited photosynthetic cells [20,21]. This interpretation is also consistent with our observation that delayed fluorescence is lost in Ca^{2+}-depleted cells. A very rapid recombination of separated charges in Ca^{2+}-depleted cells could also account for a loss in ms-delayed light, but this interpretation is unlikely since the rate of reoxidation of photoreduced Q was not significantly altered by Ca^{2+} depletion (fig.4).

The altered light emission characteristics resulting from Ca^{2+} depletion return to their normal behavior upon readdition of Ca^{2+} to the cell culture. Full recovery requires that O_2 evolution capacity is not allowed to fall completely to zero before Ca^{2+} is added. Perhaps secondary permanent photoinactivation [9] occurs in totally inactive cells.

A role for Ca^{2+} in PS II-catalyzed electron flow in isolated chloroplasts has been proposed [22]. Our results suggest that Ca^{2+} are required for photochemistry of PS II in intact cells as well. We propose that Ca^{2+} may be involved in a primary process of photosynthesis.

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