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Effects of Oxygen and Photosynthesis Carbon Cycle Reactions on Kinetics of P700 Redox Transients in Cyanobacterium *Arthrospira platensis* Cells

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Abstract—Effects of oxygen and photosynthesis and respiration inhibitors on the electron transport in photosystem I (PSI) of the cyanobacterium *Arthrospira platensis* cells were studied. Redox transients of P700 were induced by illumination at 730 nm and monitored as kinetics of the absorption changes at 810 nm; to block electron influx from PSII, the measurements were performed in the presence of 30 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). Inhibitors of terminal oxidases (potassium cyanide and pentachlorophenol) insignificantly influenced the fast oxidation of P700 under aerobic conditions, whereas removal of oxygen significantly decelerated the accumulation of $P700^+$. In the absence of oxygen the slow oxidation of P700 observed on the first illumination was accelerated on each subsequent illumination, suggesting an activation of the carbon cycle enzymes. Under the same conditions, pentachlorophenol (an uncoupler) markedly accelerated the P700 photooxidation. Under anaerobic conditions, potassium cyanide (an inhibitor of carbon dioxide assimilation) failed to influence the kinetics of redox transients of P700, whereas iodoacetamide (an inhibitor of NADP(H)-glyceraldehyde-3-phosphate dehydrogenase) completely prevented the photooxidation of P700. Thus, the fast photooxidation of P700 in the *A. platensis* cells under aerobic conditions in the presence of DCMU was caused by electron transport from PSI onto oxygen, and complicated transient changes in the P700 photooxidation kinetics under anaerobic conditions (in the presence of DCMU) were due to involvement of $NADP^+$ generated during the reducing phase of the carbon cycle.

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Key words: anaerobiosis, reducing phase of Calvin's cycle, inhibitors, kinetics of the P700 redox transients, $NADP^+$, photosystem I

Cyanobacteria are obligate phototrophs capable of oxygenic photosynthesis, which includes a light stage occurring in membranes with involvement of two photosystems (PS) and a dark stage catalyzed by enzymes of the Calvin carbon cycle [1]. Cyanobacteria are characterized by the presence of common components in the photosynthetic and respiratory pathways of electron transport: the pool of plastoquinones (PQ), cytochromes b_6f, and cytochrome b_{553} [2, 3]. The PQ pool can be reduced both by PSII of the photosynthetic chain and by NADP(H)-dehydrogenase complex of the respiratory chain, whereas cytochromes can be oxidized not only by PSI but also by the respiratory cytochrome-dependent oxidase. NADP(H) generated during the linear transport of electrons through PSI is oxidized in the course of different reactions: during the dark fixation of carbon dioxide [4], the cyclic transport of electrons around PSI [5, 6], and also during oxygen reduction with involvement of flavoproteins [7]. The multiplicity of the electron transport pathways in cyanobacteria makes it difficult to elucidate mechanisms of its regulation. Kinetics of redox transients of P700, which is the primary electron donor of PSI, will

Abbreviations: DBMIB) 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCMU) 3-(3,4-dichlorophenyl)-1,1-dimethylurea; GAPDH) glyceraldehyde-3-phosphate dehydrogenase; IAA) iodoacetamide; KCN) potassium cyanide; PCP) pentachlorophenol; P700 ($P700^+$)) the primary electron donor of the photosystem I in the reduced (oxidized) state; PQ) plastoquinone; 3-PGA (1,3-PGA)) 3-phosphoglyceric acid (1,3-phosphoglyceric acid); PSI (PSII)) photosystem I (photosystem II).

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be determined by the ratio between the electron influx to P700 from different sources and the electron efflux to the acceptor side of PSI.

The alkalophilic filamentous cyanobacterium *Arthrospira platensis* is especially interesting for elucidation of regulation of photosynthetic activity because it is widely used in biotechnology due to its ability to grow under extreme conditions [8]. This cyanobacterium is characterized by an unusually high value of the PSI/PSII ratio of 5.5 [9], whereas in higher plants this ratio is about unity. Excess PSI complexes not involved in electron linear transport seem to function in the cyclic electron flow [10].

The purpose of the present work was to elucidate the influence of different pathways of electron transport on kinetics of the P700 redox transients in the cyanobacterium *A. platensis*. Because the genome of this cyanobacterium has not yet been sequenced, it is impossible to obtain purposeful mutants in individual cofactors of the electron transport, as can be done in the case of the unicellular cyanobacteria *Synechocystis* sp. Therefore, different pathways of the electron transport in *A. platensis* cells were studied using inhibitors with effects established for other cyanobacteria. To avoid the effect of oxygen generated in PSII on the electron transport, the studies were performed in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), which inhibited reduction of the plastoquinone pool [11]. Moreover, anaerobic conditions allowed us to remove oxygen as an acceptor of electrons from PSI and, using inhibitors, determine the role of the carbon cycle reactions in the regulation of the P700 redox transients.

The present work has shown that, on illumination of cyanobacterium *A. platensis* cells in the presence of DCMU, the contribution of terminal oxidases to the electron efflux from the transport chain is insignificant and oxygen is the main acceptor of electrons from PSI. Complicated P700 transient phenomena under anaerobic conditions are determined by the influence of reducing phase reactions of the photosynthesis carbon cycle on the electron transport through PSI.

MATERIALS AND METHODS

The filamentous cyanobacterium *Arthrospira* (formerly *Spirulina*) *platensis* strain P511 was obtained from the Collection of Algae and Photosynthetic Bacteria of the Institute of Plant Physiology, Russian Academy of Sciences (Moscow). The cells were cultured at 30°C and illumination intensity of 30 $\mu E \cdot m^{-2} \cdot sec^{-1}$ on modified Zaruk medium (pH 9.6) [12] in 250-ml Erlenmeyer flasks. For measurements, 6-8-day-old cells were used, which were collected by centrifugation at 3000 rpm for 10 min and resuspended in fresh medium. Before measurements, the cells were maintained in the light (20 $\mu E \cdot m^{-2} \cdot sec^{-1}$) for 1 h.

The light-induced changes in the absorption at 810 nm (as compared to 870 nm) were recorded with a PAM-101 pulse fluorimeter equipped with an ED-P700DW double-wavelength device for P700 measurement (Walz, Germany) according to the modified method described in [13]. The actinic light of 730 nm with intensity of 300 $\mu E \cdot m^{-2} \cdot sec^{-1}$ or 620 nm with intensity of 150 $\mu E \cdot m^{-2} \cdot sec^{-1}$ was obtained from photodiodes (Walz). Light saturation curves of the P700 oxidation were recorded in actinic light with wavelength $\lambda > 680$ nm, maximal intensity of 100 $\mu E \cdot m^{-2} \cdot sec^{-1}$, from a halogen lamp and using 2-mm RG 695 filters (Schott, Germany) and a Balzers (Lichtenstain) heat filter. To obtain light with $\lambda > 680$ nm of lower intensity, a set of neutral filters was used. All measurements of the P700 redox transients were performed in the presence of 30 μM DCMU at chlorophyll concentration of 20 μg/ml in a cuvette 1 mm thick. To attain anaerobiosis in the cuvette, the suspension of *A. platensis* cells was supplemented with 10 mM glucose, glucose oxidase (24 U/ml), and catalase (400 U/ml); then the cuvette was placed into a thermostat into the dark at 30°C for 10 min. Under these conditions, anaerobiosis was attained in 5-7 min and retained in the cuvette for no less than 40 min on illumination with the actinic light; this was monitored with a Clark-type electrode (Oxygraph; Hansatech, Germany). Concentrations of the inhibitors used (2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB), potassium cyanide (KCN), pentachlorophenol (PCP), iodoacetamide (IAA)) are presented in the figure legends.

The rates of oxygen evolution in the light and oxygen consumption in the dark were measured at 30°C with the Clark electrode; the intensity of the actinic light from an incandescence lamp was 2500 $\mu E \cdot m^{-2} \cdot sec^{-1}$; the chlorophyll concentration in the chamber was 30 μg/ml. The assimilation rate of carbon dioxide by the *A. platensis* cells was measured by the rate of ^{14}C incorporation (from $NaH^{14}CO_3$) into ethanol- and water-soluble products of photosynthesis [14].

RESULTS

Effects of photosynthesis and respiration inhibitors on kinetics of the P700 redox transients in the presence of oxygen. On illumination of *A. platensis* cells with 620 nm light absorbed by both photosystems, complicated transient phenomena occurred in the P700 photooxidation kinetics in the absence of DCMU: a fast oxidation of P700, then a partial reduction of $P700^+$ caused by the electron influx from the PSII, and, finally, a slow oxidation of P700 to a stationary level (Fig. 1a). The complicated kinetics was determined by the ratio between the rates of the electron influx to P700 from the pool of reduced PQ (through the cytochrome b_6f complex and cytochrome c_{553}) and the electron efflux from the PSI.

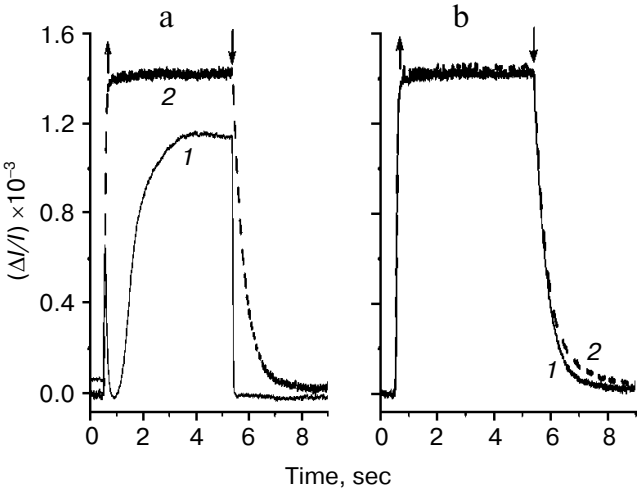

Fig. 1. Kinetics of light-induced changes in absorption at 810 nm of *A. platensis* cells under aerobic conditions: a) in the absence of DCMU: actinic light 620 nm, 150 $\mu E \cdot m^{-2} \cdot sec^{-1}$ (*1*) or 730 nm, 300 $\mu E \cdot m^{-2} \cdot sec^{-1}$ (*2*); b) in the absence (*1*) and presence of 30 μM DCMU (*2*), actinic light 730 nm, 300 $\mu E \cdot m^{-2} \cdot sec^{-1}$. Arrows ↑ and ↓ indicate the switching on and off the light, respectively, here and in the other figures.

Under these conditions, a considerable influx of electrons from PSII to PSI prevented a complete oxidation of P700 to the level resulting on the cell illumination by far red light of 730 nm (Fig. 1a). During preferential excitation at 730 nm of PSI, which is abundant in *A. platensis* cells [9], $P700^+$ was rapidly accumulated. On extinguishing the light, $P700^+$ was rather rapidly reduced by the electron influx from the donor side of PSI. On the cell illumination with the light of 620 nm, the rate of $P700^+$ dark reduction was higher ($\tau_{1/2}$ ~ 50 msec) than on illumination with far red light ($\tau_{1/2}$ ~ 350 msec) due to the more complete reduction of the PQ pool by PSII.

Kinetic curves of changes in the absorption at 810 nm of the *A. platensis* cells induced by the light of 730 nm in the presence and absence of DCMU are rather similar (Fig. 1b). A slight acceleration of the dark reduction of $P700^+$ in the cells in the absence of DCMU seemed to be associated with a partial excitation of PSII by the actinic light (730 nm). The rapid oxidation of P700 and deceleration of the dark reduction of $P700^+$ on addition of DBMIB (+DCMU) to the *A. platensis* cells (Fig. 2, curve *2*) was associated with the inhibition by DBMIB (as an antagonist of PQ) of the linear electron transfer and the cyclic electron transport around PSI on the section between the PQ pool and the cytochrome b_6f complex [11].

Because terminal oxidases compete with P700 for electrons from PQ [15, 16], it was interesting to investigate the influence of KCN (an inhibitor of terminal oxidases) on the photoinduced redox transients of P700. Incubation of the cells for 2-3 min in the presence of KCN and DCMU slightly slowed the P700 photooxidation and accelerated the $P700^+$ dark reduction (Fig. 2, curve *3*). Because of the absence of plastocyanin in *A. platensis* cells [8], KCN did not inhibit the electron transport in the donor side of PSI. It seemed that the heme of the cytochrome, which in the absence of plastocyanin was involved in the electron transport on this section of the chain, was unavailable for KCN owing to the structure of the protein moiety of the cytochrome molecule. In the absence of DCMU, 10 mM KCN strongly suppressed the photosynthetic release of oxygen and 90% decreased the fixation of CO_2, which was evaluated by the assimilation of $^{14}CO_2$. Such an effect of cyanide was associated with inhibition of ribulose 1,5-diphosphate carboxylase and(or) carboanhydrase [17-19]. In the presence of DCMU only the cyclic electron transport around the PSI could function in the far red light [20-22]; therefore, changes in the P700 redox transients observed on addition of KCN + DCMU seemed to be unassociated with the influence of KCN on both the donor and acceptor sides of PSI. Most likely, the cyanide-induced inhibition of the electron transport through oxidases in the cytoplasmic and thylakoid membranes led to accumulation of reduced electron carriers between the photosystems, which, in turn, could slow down P700 photooxidation. The accumulation of reduced PQ in the presence of cyanide was found in the cells of the cyanobacterium *Fremiella diplosiphon* [23]. Therefore, the faster dark reduction of $P700^+$ in the presence of DCMU + KCN (compare with DCMU alone; Fig. 2, curves *1* and *3*) seemed to be caused by an effective donation of electrons from PQ and the cytochromes.

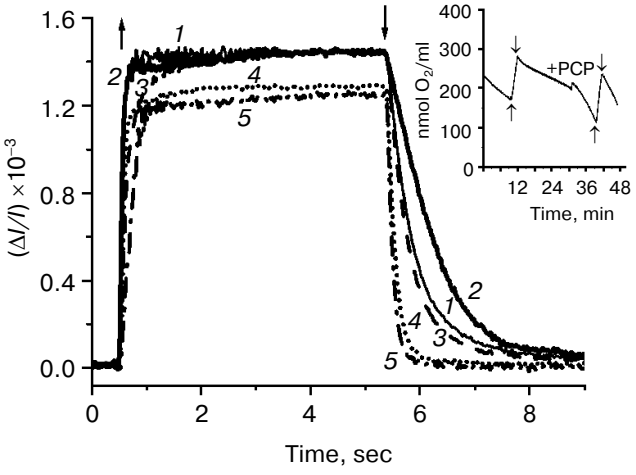

Fig. 2. Kinetics of the light-induced changes in the absorption at 810 nm of *A. platensis* cells under aerobic conditions in the presence of 30 μM DCMU (*1*), 30 μM DCMU + 100 μM DBMIB (*2*), 30 μM DCMU + 8 mM KCN (*3*), 30 μM DCMU + 1 mM PCP (*4*), or 30 μm DCMU + 1 mM PCP + 8 mM KCN (*5*). The actinic light 730 nm, 300 $\mu E \cdot m^{-2} \cdot sec^{-1}$. Inset: evolution of oxygen in the light and oxygen consumption in the dark by the *A. platensis* cells in the presence of 1 mM PCP, white actinic light 2500 $\mu E \cdot m^{-2} \cdot sec^{-1}$.

The combined treatment with DCMU and pentachlorophenol (PCP), an inhibitor of the alternative cytochrome *bd* oxidase [15], decreased the accumulation of $P700^+$ and enhanced the rate of its dark reduction, as compared with the pair of KCN + DCMU (Fig. 2, curve *4*). These changes could be caused by both the reduction of the PQ pool due to inhibition of the alternative oxidase and the uncoupling effect of PCP which, at the concentration of 1 mM, prevented acidification of the intra-thylakoid space and accelerated the oxidation of reduced PQ by the cytochrome b_6f complex [24, 25]. It should be noted that kinetics of the P700 photooxidation in *A. platensis* cells incubated in the presence of DCMU concurrently with KCN and PCP were virtually the same as the kinetics measured in the presence of DCMU and PCP alone (Fig. 2, curves *4* and *5*). As judged from the rate of oxygen evolution by the *A. platensis* cells in the presence of PCP (but without DCMU), at 1 mM this inhibitor did not suppress the linear photosynthetic transport of electrons, as indicated by the light-dependent oxygen release under these conditions (Fig. 2, inset). Thus, the contribution of terminal oxidases to the electron efflux from the transport chain in the *A. platensis* cells revealed using KCN was insignificant; changes in the P700 photooxidation kinetics in the presence of PCP were likely to be mainly due to the strong uncoupling effect of this inhibitor.

Light saturation curves of P700 redox transients in the presence of electron transport inhibitors. To assess the efficiency of the electron influx to P700 determined by the reduction level of the PQ and cytochrome pool, the dependence of the P700 photooxidation on intensity of the actinic light with $\lambda > 680$ nm was studied in the presence of different inhibitors (Fig. 3). The 50% oxidation of P700 in the presence of DCMU alone was observed at the actinic light intensity of 10-12.5% of the maximum (curve *1*), and at the markedly lower intensity (5.5-7.5%) in the presence of the DCMU + DBMIB pair (curve *2*). Considering the DBMIB-caused inhibition of the electron transport from PQ to the PSI, it was suggested that in the presence of DBMIB the donor side of the PSI should be reduced poorly. The 50% accumulation of $P700^+$ in the presence of DCMU + KCN (curve *3*) or DCMU + PCP (curve *4*) occurred under more intense illumination: 20-30 or 45-52% of the maximum intensity, respectively. It seemed that in these cases electron carriers in the donor side of PSI were more reduced than in the presence of DCMU alone or the DCMU + DBMIB pair; therefore, $P700^+$ reduction occurred with higher rate.

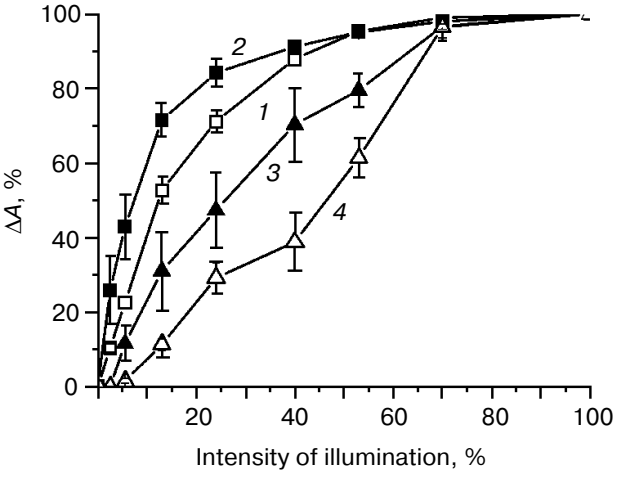

Fig. 3. Light saturation curves of P700 redox changes in *A. platensis* cells in the presence of 30 μM DCMU (*1*), 30 μM DCMU + 100 μM DBMIB (*2*), 30 μM DCMU + 8 mM KCN (*3*), 30 μM DCMU + 1 mM PCP (*4*); the maximal intensity of the actinic light ($\lambda > 680$ nm) was 100 $\mu E \cdot m^{-2} \cdot sec^{-1}$. ΔA is the ratio of the absorption change measured at lower intensity to the absorption change measured at maximal intensity. The curves *1-4* present the means of five independent experiments.

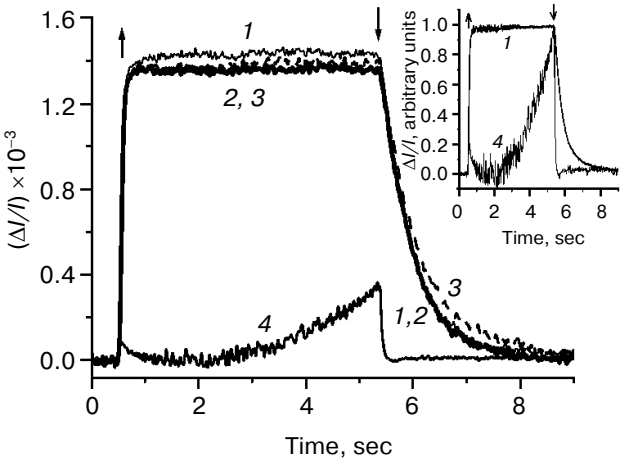

Fig. 4. Kinetics of light-induced changes in the absorption at 810 nm of *A. platensis* cells in the presence of 30 μM DCMU under aerobic (*1-3*) and anaerobic (*4*) conditions; the actinic light 730 nm, 300 $\mu E \cdot m^{-2} \cdot sec^{-1}$. Curve (*1*) was obtained on the first switching on the actinic light, curve (*2*) on the second switching on of the light after a 30-sec dark interval, curve (*3*) on the switching on of the light after 1 min of darkness. Inset: normalized kinetics of the P700 redox changes in the presence of DCMU under aerobic (*1*) and anaerobic conditions (*4*).

Effects of electron transport inhibitors on kinetics of P700 redox transients under anaerobic conditions. The removal of oxygen by the enzymatic system (glucose, glucose oxidase, and catalase) from the culture medium containing the *A. platensis* cells in the presence of DCMU strongly decelerated P700 oxidation, decreased the level of $P700^+$, and accelerated its dark reduction (Fig. 4). Incubation of the cyanobacterium cells for 10 min in the presence of DCMU and 10 mM glucose under aerobic conditions had no influence on kinetics of the photoinduced changes in the absorption of P700. The strong deceleration of the P700 oxidation and acceleration of the dark reduction of $P700^+$ suggested an accumulation of reduced electron carriers as a result of inhibition of the

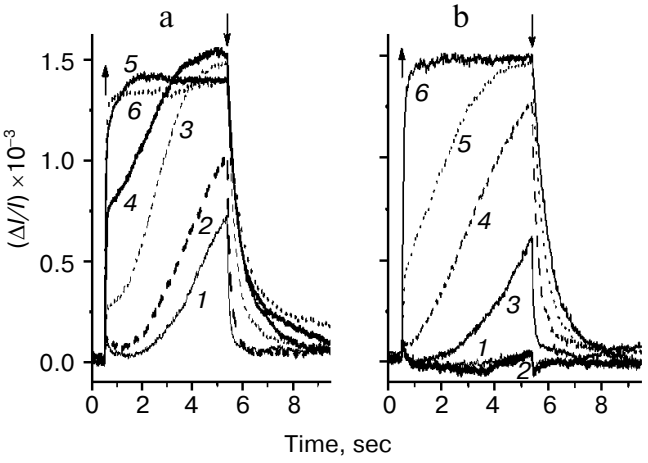

Fig. 5. Kinetics of light-induced changes in absorption at 810 nm of *A. platensis* cells under anaerobic conditions in the presence of 30 μM DCMU; actinic light 730 nm, 300 $\mu E \cdot m^{-2} \cdot sec^{-1}$. a) Before measurements, the cells were illuminated at 20 $\mu E \cdot m^{-2} \cdot sec^{-1}$ for 1 h; b) before measurements, the cells were kept in the dark for 2 h. Here and in Fig. 6: *1*) first illumination; *2*) repeated illumination 30 sec after the first one; *3*) illumination 1 min after the second one; *4*) illumination 3 min after the third one; *5*) illumination after 15-sec exposition with a background illumination at 730 nm; *6*) illumination after admission of air into the cuvette.

electron efflux from PSI to oxygen. These findings confirmed the role of oxygen as an acceptor of electrons from the PSI, which had been earlier shown in cells of *Synechocystis* sp. [26, 27].

The kinetics of P700 oxidation induced by the light of 730 nm in the presence of oxygen (and DCMU) were almost unchanged on consecutive switching on of the light alternating with dark intervals (Fig. 4, curves *1-3*) and were sharply slowed down in the absence of oxygen (curve *4*). The pattern was quite different when the *A. platensis* cells were consecutively illuminated with light of 730 nm under anaerobic conditions (in the presence of DCMU). Each illumination of the cells enhanced the rate of the P700 oxidation; the longer the dark interval between the illuminations, the faster the oxidation of P700 (Fig. 5a, curves *1-4*). The oxidation rate was the highest on the preliminary illumination of the cells with the far red light of 730 nm within 15 sec (Fig. 5a, curve *5*). After air was admitted into the cuvette with the cells kept under anaerobic conditions, the actinic light of 730 nm caused a very fast oxidation of P700 (Fig. 5a, curve *6*) that suggested oxygen functioning as an acceptor from the PSI or intersystem carriers [28]. These findings indicated that under anaerobic conditions the electron efflux from the PS1 mainly occurred via the light-activated linear reduction of the acceptor side of PSI including $NADP^+$, which as NADP(H) either could be used in the Calvin cycle reactions [4] or reduce the pool of PQ [6].

The above-presented data were obtained on cells that were maintained in the light (20 $\mu E \cdot m^{-2} \cdot sec^{-1}$) for 1 h before measurements. If before the measurements the *A. platensis* cells were kept in the dark for 2 h and then placed under anaerobic conditions in the presence of DCMU, the rate of the P700 oxidation on consecutive illuminations by the far red light was considerably lower (Fig. 5b, curves *1-5*). Differences between the cells maintained in the light and in the dark could be caused by inhibition of reactions responsible for carbon dioxide fixation in the dark and also by a possible reduction of PQ by NADP(H) [5, 6].

Based on the totality of the findings, it was suggested that under anaerobic conditions and on reduction of the PSI donor side the far red light should trigger cyclic electron transport around PSI, which resulted in the activation of the photosynthesis carbon cycle reactions [21, 29]. This activation determines the lag period in the P700 oxidation (Fig. 5). Therefore, it was reasonable to investigate the effect of KCN as an inhibitor of the carbon dioxide dark fixation on the P700 photooxidation in *A. platensis* cells under anaerobic conditions in the presence of DCMU. It was found that 8 mM KCN had virtually no effect on processes determining the P700 oxidation in the *A. platensis* cells (Fig. 6a), although this concentration of

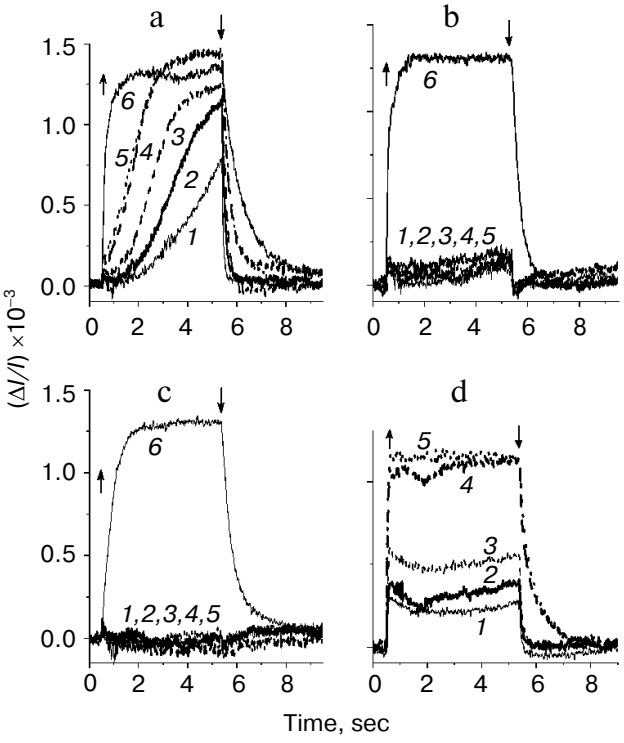

Fig. 6. Kinetics of light-induced changes in absorption at 810 nm of *A. platensis* cells under anaerobic conditions: a) in the presence of 30 μM DCMU + 8 mM KCN; b) in the presence of 30 μM DCMU + 8 mM IAA; c) in the presence of 30 μM DCMU + 8 mM KCN + 8 mM IAA; d) in the presence of 30 μM DCMU + 1 mM PCP; the actinic light 730 nm, 300 $\mu E \cdot m^{-2} \cdot sec^{-1}$. Before measurements, the cells were kept in the light 20 $\mu E \cdot m^{-2} \cdot sec^{-1}$ for 1 h.

cyanide had to effectively inhibit the activity of ribulose 1,5-diphosphate carboxylase and, possibly, carboanhydrase [17, 18]. Iodoacetamide (IAA), an inhibitor of the Calvin cycle enzymes, including glyceraldehyde phosphate dehydrogenase (GAPDH) [30, 31] effectively inhibited the P700 oxidation under anaerobic conditions (in the presence of DCMU) in the far red light (Fig. 6b). The admission of air into the cuvette containing the *A. platensis* cells accelerated the P700 photooxidation. The P700 oxidation was virtually completely suppressed by the simultaneous treatment with IAA and KCN (Fig. 6c). It seems that in addition to the NADP(H) involvement in reactions of the carbon cycle reducing phase, there is another, less pronounced pathway of NADP(H) reoxidation, which is inhibited by KCN. Consequently, kinetics of the P700 photooxidation under anaerobic conditions is mainly controlled by functioning of the reducing phase of the Calvin cycle during which $NADP^+$ is generated.

In addition to NADP(H), the reduction of 3-phosphoglyceric acid (3-PGA) to phosphoglyceraldehyde also needed ATP, which significantly activated this reaction because it was necessary for the preceding phosphorylation of 3-PGA to 1,3-phosphoglyceric acid (1,3-PGA) (the substrate of GAPDH). An effective uncoupler (PCP) had to influence the kinetics of this process and indirectly the kinetics of P700 photooxidation. In fact, on the measurement of P700 photooxidation in the *A. platensis* cells under anaerobic conditions in the presence of DCMU and PCP only the fast component of the P700 oxidation was observed, whereas the lag-period and slow component disappeared (Fig. 6d). Thus, just the phosphorylation reaction seemed to be responsible for the slow component of the P700 oxidation. On consecutive illuminations with light of 730 nm of the *A. platensis* cells under anaerobic conditions in the presence of DCMU and PCP the accumulation of $P700^+$ increased (Fig. 6d); the longer the interval between the illuminations, the higher the accumulation. This suggested the light-induced activation of GAPDH, which occurred even in the presence of PCP and was not associated with the presence of ATP. It seemed that the fast component of the P700 photooxidation (in the presence of PCP) was associated with reduction of the available $NADP^+$ or $NADP^+$ that is accumulated in the light during the same reaction catalyzed by GAPDH [32]. The deceleration of the $P700^+$ dark reduction in the *A. platensis* cells in the presence of PCP indicated a rapid exhaustion of the electron equivalent pool in the donor side of PSI.

Thus, kinetics of the P700 oxidation recorded on the illumination of *A. platensis* cells by far red light under anaerobic conditions in the presence of DCMU were caused by reactions of the carbon cycle where NADP(H) was expended and $NADP^+$ (PSI acceptor) was generated. Under these conditions, electrons for reduction of $NADP^+$ came from carriers of the PSI donor side, which was more reduced due to absence of oxygen and involvement of the NADP(H) dehydrogenase complex in donation of electrons from stromal reductants.

DISCUSSION

The findings have shown that the complicated kinetics of P700 redox transients under anaerobic conditions is caused by influences of the Calvin cycle reactions. Effects of these reactions on P700 cannot be detected under aerobic conditions in the presence of DCMU because oxygen is an effective acceptor of electrons from PSI. The kinetics of P700 photooxidation in *A. platensis* cells incubated in the presence of oxidase inhibitors (KCN and PCP) and DCMU under usual conditions were unlike the kinetics of P700 photooxidation under anaerobiosis (with DCMU); therefore, it was concluded that oxygen should be involved in oxidation of the acceptor side of PSI. In fact, P700 was oxidized rapidly on the admission of air into the cell suspension maintained under anaerobic conditions in the presence of DCMU alone or KCN + DCMU (Figs. 5 and 6a), which indicated a rapid cyanide-independent reduction of oxygen in PSI. Such a reduction of oxygen can occur in cyanobacteria with involvement of the A-type flavoproteins, which form a complex of electron transport from NADP(H) to oxygen with production of water [7].

As distinguished from usual conditions in air, the rate of P700 oxidation increased on each consecutive illumination under anaerobic conditions in the presence of DCMU; the longer the interval between illuminations, the higher the rate of P700 oxidation (compare Figs. 4 and 5). This finding indicated that under anaerobic conditions appearance of acceptors for the PSI was activated by light reactions and seemed to be associated with the carbon cycle of photosynthesis. This hypothesis of activation proposed in [27] was confirmed by using IAA (an inhibitor of carbon dioxide assimilation), which suppressed P700 oxidation under anaerobic conditions (Fig. 6b). Note, that another inhibitor of assimilation of carbon dioxide, KCN, had almost no effect on P700 photooxidation kinetics under anaerobic conditions (Figs. 5 and 6a). This difference was likely to be associated with different action mechanisms of these inhibitors [18, 19]. In addition to suppression of the carboxylation phase, IAA, as differentiated from KCN, inhibits SH groups of many enzymes including GAPDH [30, 31], which is the main enzyme of the reducing phase of the Calvin cycle. 3-PGA is not only a substrate of the reducing phase in the Calvin cycle but also intermediate during glycolysis. The pathways of glucose assimilation by cyanobacteria are multiform, but enzymes of glycolysis (the Embden–Meyerhof–Parnas pathway) are always involved in fermentation [1]. In *A. platensis* cells, fermentation always occurs by the pathway of anaerobic degradation of endogenous glycogen through pyruvate [33], which suggests the involvement of glycoly-

sis enzymes. Thus, 3-PGA is a central metabolite of the carbohydrate metabolism of cyanobacteria.

Earlier, "modules" of thylakoid membranes presenting a synchronized system of the dark and light reactions of photosynthesis were isolated from *Anacystis nidulans* cells; most components of these "modules" were retained even after centrifugation in a sucrose density gradient [34]. The GAPDH activity of cyanobacteria is also shown to be regulated by association/dissociation of this enzyme complex with phosphoribulose and the CP12 protein depending on the NAD(H)/NADP(H) ratio, and this ratio is supposed to be important *in situ* [32]. The data obtained on *A. platensis* cells are consistent with this hypothesis. The suppression by IAA (an inhibitor of carbon dioxide assimilation) of the P700 photooxidation under anaerobic conditions and disappearance of the slow component of the kinetics in the presence of the uncoupler PCP allowed us to conclude that the kinetics of the P700 oxidation of the *A. platensis* cells under anaerobic conditions (in the presence of DCMU) should represent reduction of $NADP^+$, the quantity of which increased on light activation of the Calvin cycle reducing phase reactions.

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REFERENCES

1. Stal, L. J., and Moezelaar, R. (1997) *FEMS Microbiol. Rev.*, **21**, 179-211.
2. Peschek, G. A. (1987) in *The Cyanobacteria* (Fay, P., and van Baalen, C., eds.) Elsevier, Amsterdam, pp. 119-161.
3. Schmetterer, G. (1994) in *The Molecular Biology of Cyanobacteria* (Bryant, D. A., ed.) Kluwer Academic Publishers, Dordrecht, pp. 409-435.
4. Ogawa, T. (1992) in *Research in Photosynthesis* (Murata, N., ed.) Vol. III, Kluwer Academic Publishers, Dordrecht, pp. 831-834.
5. Mi, H., Endo, T., Schreiber, U., Ogawa, T., and Asada, K. (1992) *Plant Cell Physiol.*, **33**, 1233-1237.
6. Bukhov, N. G., and Carpantier, R. (2004) *Photosynth. Res.*, **82**, 17-33.
7. Helman, Y., Tchernov, D., Reinhold, L., Shibata, M., Ogawa, T., Schwarz, R., Ohad, I., and Kaplan, A. (2003) *Curr. Biol.*, **13**, 230-235.
8. Vonshak, A. (1997) in *Spirulina platensis (Arthrospira)* (Vonshak, A., ed.) Taylor & Francis, London, pp. 43-65.
9. Rakhimberdieva, M. G., Boichenko, V. A., Karapetyan, N. V., and Stadnichuk, I. N. (2001) *Biochemistry*, **40**, 15780-15788.
10. Berry, S., Bolychevtseva, Y. V., Roegner, M., and Karapetyan, N. V. (2003) *Photosynth. Res.*, **78**, 67-76.
11. Trebst, A. (1980) *Meth. Enzymol.*, **69**, 675-715.
12. Terekhova, I. V., Chernyad'ev, I. I., and Doman, N. G. (1983) *Prikl. Biokhim. Mikrobiol.*, **19**, 473-480.
13. Schreiber, U., Klughammer, C., and Neubauer, C. (1988) *Z. Naturforsch.*, **43c**, 686-698.
14. Bolychevtseva, Yu. V., Mazhorova, L. E., Terekhova, I. V., Egorova, E. A., Shugaev, A. G., Rakhimberdieva, M. G., and Karapetyan, N. V. (2003) *Appl. Biochem. Microbiol.*, **39**, 503-508.
15. Pils, D., Gregor, W., and Schmetterer, G. (1997) *FEMS Microbiol. Lett.*, **152**, 83-88.
16. Paumann, M., Regelsberger, G., Obinger, C., and Peschek, G. A. (2005) *Biochim. Biophys. Acta*, **1707**, 231-253.
17. Wishnick, M., and Lane, M. D. (1969) *J. Biol. Chem.*, **244**, 55-59.
18. Forti, G., and Gerola, P. (1977) *Plant Physiol.*, **59**, 859-862.
19. Belknap, W. R., and Togasaki, R. K. (1982) *Plant Physiol.*, **70**, 469-475.
20. Bendall, D. E., and Manasse, R. S. (1995) *Biochim. Biophys. Acta*, **1229**, 23-38.
21. Joliot, P., and Joliot, A. (2002) *Proc. Natl. Acad. Sci. USA*, **99**, 10209-10214.
22. Joliot, P., and Joliot, A. (2006) in *Photosystem I: The Light-Driven Plastocyanin/Ferredoxin/Oxidoreductase* (Golbeck, J. H., ed.) *Series Advances in Photosynthesis and Respiration*, Vol. 24, Springer, Dordrecht, pp. 639-656.
23. Schubert, H., Matthijs, H. C. P., and Mur, L. R. (1995) *Photosynthetica*, **31**, 517-527.
24. Tissut, M., Taillandier, G., Ravanel, P., and Benoit-Guyod, J. L. (1987) *Ecotoxicol. Environ. Saf.*, **13**, 32-42.
25. Gravance, C. G., Garner, D. L., Miller, M. G., and Berger, T. (2003) *Toxicol. in vitro*, **173**, 253-257.
26. Trubitsin, B. V., Mamedov, M. D., Vitukhnovskaya, L. A., Semenov, A. Y., and Tikhonov, A. N. (2003) *FEBS Lett.*, **544**, 15-20.
27. Trubitsin, B. V., Ptushenko, V. V., Koksharova, O. A., Mamedov, M. D., Vitukhnovskaya, I. A., Grigor'ev, I. A., Semenov, A. Y., and Tikhonov, A. N. (2005) *Biochim. Biophys. Acta*, **1708**, 238-249.
28. Khorobrykh, S. A., and Ivanov, B. N. (2002) *Photosynth. Res.*, **71**, 209-219.
29. Munekage, Y., Hashimoto, M., Miyake, C., Tomizawa, K.-I., Endo, T., Tasaka, M., and Shikanai, T. (2004) *Nature*, **429**, 579-582.
30. Peschek, G. A. (1978) *Arch. Microbiol.*, **119**, 313-322.
31. Lebreton, S., Graciet, E., and Gontero, B. (2003) *J. Biol. Chem.*, **278**, 12078-12084.
32. Tamoi, M., Miyazaki, T., Fukamizo, T., and Shigeoka, S. (2005) *The Plant J.*, **42**, 504-513.
33. Aoyama, K., Uemura, I., Miyake, I., and Asada, Y. (1997) *J. Ferment. Bioeng.*, **83**, 17-20.
34. Dani, D. N., and Sainis, J. K. (2005) *Biochim. Biophys. Acta*, **1669**, 43-52.