ISSN 0006-2979, Biochemistry (Moscow), 2011, Vol. 76, No. 4, pp. 427-437. © Pleiades Publishing, Ltd., 2011.
Original Russian Text © Yu. V. Bolychevtseva, I. V. Elanskaya, N. V. Karapetyan, 2011, published in Biokhimiya, 2011, Vol. 76, No. 4, pp. 525-536.
Originally published in Biochemistry (Moscow) On-Line Papers in Press, as Manuscript BM10-365, March 6, 2011.

Regulation of Cyclic Electron Transport through Photosystem I in Cyanobacterium *Synechocystis* sp. PCC 6803 Mutants Deficient in Respiratory Dehydrogenases

Yu. V. Bolychevtseva[1]*, I. V. Elanskaya[2], and N. V. Karapetyan[1]

[1]*Bach Institute of Biochemistry, Russian Academy of Sciences, Leninsky pr. 33, 119071 Moscow, Russia; fax: (495) 954-2732; E-mail: bolychev@inbi.ras.ru; nkarap@inbi.ras.ru*
[2]*Department of Genetics, Faculty of Biology, Lomonosov Moscow State University, 119992 Moscow, Russia; fax: (495) 939-2957; E-mail: ivelanskaya@mail.ru*

Received November 29, 2010
Revision received January 14, 2011

Abstract—The rate of PSI mediated cyclic electron transport was studied in wild type and mutant cells of *Synechocystis* sp. PCC 6803 deficient in NDH-1 (M55) or succinate dehydrogenase (SDH^-) that are responsible for the dark reduction of the plastoquinone pool. Kinetics of P700 photooxidation and $P700^+$ dark reduction in the presence of $5 \cdot 10^{-5}$ M 3-(3,4-dichlorophenyl)-1,1-dimethylurea have been registered as light induced absorbance changes at 810 nm resulting from illumination of cells with 730-nm actinic light for 1 sec. It is shown that in the absence of dehydrogenases the rate of dark reduction of $P700^+$ in both mutants did not decrease but even increased in NDH-1-less mutant cells as compared with the rate in wild type cells. Dibromothymoquinone drastically reduced the rate of $P700^+$ dark reduction both in wild type and in mutant cells. Thus, the cyclic electron transfer from ferredoxin through the plastoquinone pool to $P700^+$, which is independent from dehydrogenases, takes place in all the types of cells. Preillumination of cells of wild type and both mutants for 30 min or anaerobic conditions resulted in delay of P700 photooxidation and acceleration of $P700^+$ dark reduction, while the level of photosynthesis and respiration terminal acceptors ($NAD(P)^+$ and oxygen) decreased. It appears that the rate of P700 photooxidation and $P700^+$ dark reduction in cyclic electron transport in *Synechocystis* wild type and mutant cells is determined by the level of $NADP^+$ and oxygen in stroma. A possible approach to evaluation of the levels of these acceptors *in vivo* is proposed, based on kinetic curve parameters of P700 photoconversions induced by 730-nm light with 1-sec duration.

DOI: 10.1134/S0006297911040055

Key words: cyclic electron transport, NADPH, P700 redox transients, NDH-1, SDH

Thylakoid membranes of *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) include the photosynthetic electron transport chain complexes (photosystem 1 and photosystem 2) and respiratory chain components, such as dehydrogenases NDH-1 (primarily NADPH-dependent) and NDH-2 (NADH-dependent), succinate dehydrogenase (SDH), and terminal oxidases (Cyd, CtaI, CtaII) [1-5]. The respiratory and photosynthetic electron transport chains have common electron carriers—cytochrome b_6/f complex and the plastoquinone (PQ) pool [2, 3]. Cytoplasmic membranes form the inner boundary of the periplasmic space and contain complexes typical for the respiratory electron transport chain, such as NDH-2 dehydrogenase, succinate dehydrogenase, and terminal oxidase (presumably plastoquinol oxidase Cyd) [2, 4, 5]. The PQ pool in thylakoid membrane can be reduced either by PSII, or by respiratory dehydrogenases, and plastoquinol oxidation may be carried out not only by PSI, but also by cytochrome- and quinol-dependent oxidases. There are soluble proteins capable of redox transformations in the cytoplasmic space, common

Abbreviations: Cyd, CtaI, and CtaII are terminal oxidases; DBMIB, dibromothymoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Fd, ferredoxin; FNR, ferredoxin:NADP-oxidoreductase; FQR, ferredoxin:plastoquinone-oxidoreductase; F_v, variable part of fluorescence; NDH-1 and NDH-2 are dehydrogenases; P700 ($P700^+$), primary electron donor of PSI in reduced (oxidized) state; PQ, plastoquinone; PSI (PSII), photosystem 1 (2); SDH, succinate dehydrogenase.

* To whom correspondence should be addressed.

to the two types of membranes: ferredoxin and flavoproteins, pool of cofactors (pyridine nucleotides) common to respiration and photosynthesis, as well as a pool of respiratory substrates such as glucose and succinate.

Dark reduction of $P700^+$ after illumination of cyanobacterial cells with far-red light or with white light in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) is carried out by cyclic electron transport through photosystem 1 (PSI) [6, 7]. At least two different pathways of cyclic electron transport through PSI are known: Fd-dependent and NDH-1-dependent. In the Fd-dependent pathway, electrons from Fd are transported to the donor part of PSI by a partially characterized complex that supposedly includes FQR and perhaps FNR [8-10]. In addition, electrons can be transferred from NADPH, formed in the light, to the PQ pool via respiratory complex NDH-1 [11, 12]. The thylakoid NDH-1 dehydrogenase of cyanobacteria can use NADPH formed either in noncyclic photosynthetic electron transport or from glucose in the oxidative pentose phosphate pathway to reduce the PQ pool [11, 13, 14]. In addition, *Synechocystis* contains succinate:quinone-oxidoreductase (SDH), which can also reduce the PQ pool [4], introducing additional electrons into the cyclic flow through PSI.

Decrease in PSI electron acceptor levels ($NADP^+$ and oxygen) in chloroplasts of higher plants increases the fluorescence yield after turning off the light [15] and lowers the level of $P700^+$, which indicates the dependence of cyclic electron transport through the PSI on redox state of electron acceptor pools of PSI in the stroma [16-18]. The main pathway of PQ pool reduction by stromal reductants during cyclic electron transport through the PSI in *Synechocystis* cells remains unclear: whether NAD(P)H, located in the cell stroma reduces the PQ pool directly via the respiratory dehydrogenase, or the absence of these enzymes changes the level of reduced pyridine nucleotides in the cell stroma, which may indirectly (via the Fd-dependent pathway of electron transport) influence the speed of P700 redox transformations. To address this issue, we investigated the changes in the rates of P700 photooxidation and $P700^+$ dark reduction in cyclic electron transport in cells of mutant *Synechocystis* lacking NDH-1 dehydrogenase or succinate dehydrogenase (M55 or SDH^-, respectively). We show in the present study that the rate of P700 photooxidation and $P700^+$ dark reduction in cyclic electron transport in wild type and mutant cells of *Synechocystis* are determined not by the direct action of dehydrogenases, but indirectly by the level of $NADP^+$ and oxygen – the final PSI electron acceptors in stroma.

MATERIALS AND METHODS

Cells of wild-type cyanobacterium *Synechocystis* sp. PCC 6803 and mutants M55 (lacking NdhB-subunit of NDH-1 dehydrogenase) [19] and SDH^- (lacking succinate dehydrogenase) [4] were grown photoautotrophically for 4-5 days at 30°C under constant illumination with 50 $\mu E \cdot m^{-2} \cdot sec^{-1}$ daylight lamp and with continuous stirring in liquid mineral medium BG-11 [20]. Cells were collected by soft filtration on filters (Nalgene; pore size 0.45 μm, volume 150 ml) with a hand vacuum pump (Nalgene) and diluted with fresh medium to the desired density of the suspension. Chlorophyll concentration was determined in 80% acetone extracts according to the method described in [21]. The collected cells were kept for 30 min under light from an incandescent lamp (25 $\mu E \cdot m^{-2} \cdot sec^{-1}$) or 2-2.5 h in darkness, while the oxygen content in the polarographic cell during respiration in darkness decreased by 95% after the first hour. Since NADPH level increases after the incubation of photosynthetic cells in the light [17, 22] and decreases after incubation in the dark [11], we assumed that under these conditions the cells will vary in $NADP^+$/NADPH ratio in the stroma.

Fluorescence induction of PSII from *Synechocystis* cells and photo-induced P700 absorption changes at 810 nm (compared to 870 nm) were measured at 27°C with a PAM101 pulse fluorimeter [23]. We used an ED-P700DW dual-wavelength accessory (Walz, Germany) to the PAM101 to measure the changes in P700 absorption [24]. A controlled high power LED-lamp (Walz) was used as the light source (730 nm) of varying intensity (from 1300 to 8550 $\mu E \cdot m^{-2} \cdot sec^{-1}$). Additional illumination for 1.5 min with 680-nm light (1300 $\mu E \cdot m^{-2} \cdot sec^{-1}$) was carried out with a halogen lamp (KL 1500) with interference filter BPF 680 (Fotooptik, Russia) and thermal filter (Balzers, Liechtenstein). The intensity of the actinic light (730 nm) and the additional light (680 nm) was measured using an Optical Power Meter System (Thorlabs, Germany). All measurements of P700 redox transformations were performed in the presence of $5 \cdot 10^{-5}$ M DCMU and chlorophyll concentration of 5-7 μg/ml in a 1-cm pathlength cuvette. We added to the cell suspension glucose (10 mM), glucose oxidase (24 units/ml), and catalase (400 units/ml) to create anaerobiosis in the cuvette. Then the cuvette was thermostatted (in darkness in the presence of $5 \cdot 10^{-5}$ M DCMU) at 27°C for 10 min with a US-T accessory (Walz). Anaerobiosis was established after 5-7 min and was maintained in the cuvette for 40 min under illumination with actinic light, which was tested with a Clark electrode (Oxygraph, Hansatech, Germany). Concentrations of inhibitors (dibromothymoquinone (DBMIB) and KCN) are shown in the figures legends.

RESULTS

It was previously shown using the voltammetric method with a special Q-electrode that *Synechocystis* mutants lacking NDH-1 dehydrogenase (M55) or succinate dehydrogenase (SDH^-) have a PQ pool that is oxi-

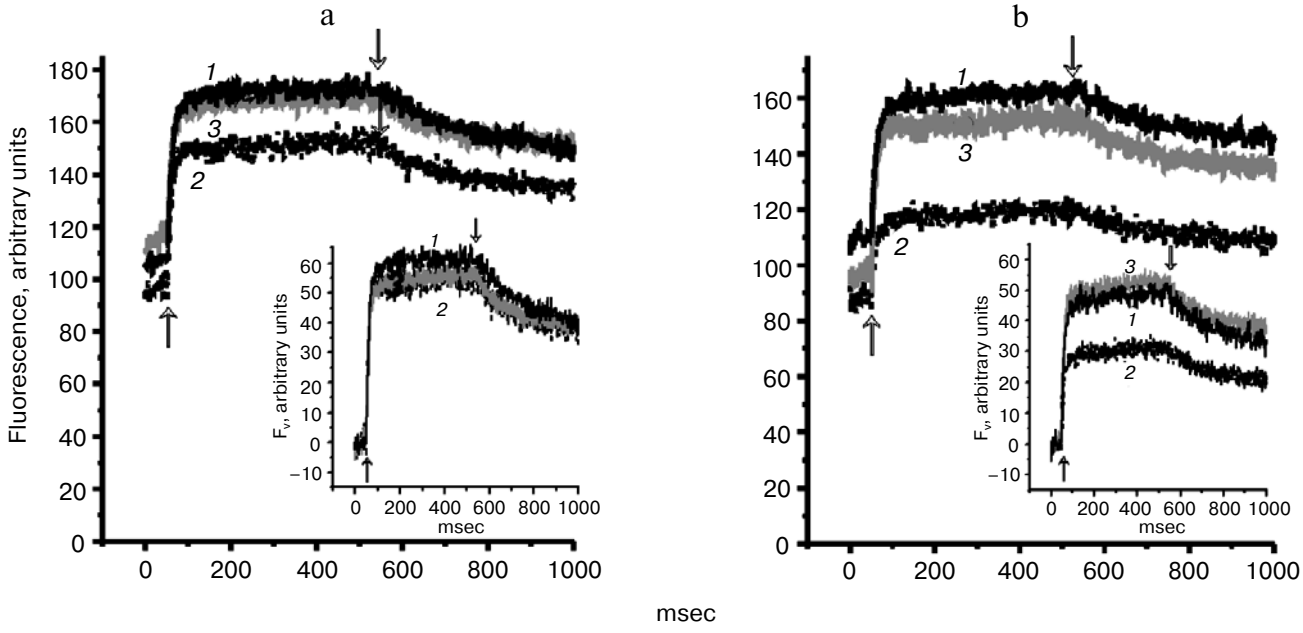


Fig. 1. Fluorescence induction of wild-type (*1*) and mutant M55 (*2*) and SDH$^-$ (*3*) cells of *Synechocystis* preilluminated for 30 min (25 μE·m^{-2}·sec^{-1}) (a) or kept in darkness for 2 h (b) in the presence of 5·10^{-5} M DCMU (620-nm actinic light (30 μE·m^{-2}·sec^{-1}); up and down arrows, turning on and off the actinic light). The insets show the variable part of fluorescence (F_v); chlorophyll content in the samples was 5 μg/ml.

dized compared with that of wild-type cells [25]. We estimated the PQ pool redox state of *Synechocystis* mutants by the ratio of the area above the fluorescence induction curve with DBMIB and ascorbate (S_{DBMIB}) to the area above the fluorescence induction curve with DCMU (S_{DCMU}) [26]. It should be noted that wild type and mutants cells kept for 30 min under white light (25 μE·m^{-2}·sec^{-1}) in the presence of DCMU are characterized by approximately the same variable fluorescence kinetics (Fig. 1a), whereas cells kept in the darkness even in the presence of DCMU differ in the variable fluorescence kinetics (Fig. 1b). Judging by the same variable fluorescence (F_v) in the presence of DCMU (Fig. 1a, inset), PSII content in the cells of wild type and mutants is not altered. Lower value of the fluorescence variable after 2 h dark incubation in wild-type cells and, especially, M55 mutant cells, in comparison with fluorescence induction in cells kept in the light (inset in Figs. 1a and 1b) may be caused by transformation of the cyanobacterial cells into state 2 characterized by low PSII activity [27]. Therefore, for comparison of PQ pool redox states in wild type cells and the two mutants, area ratios were measured in *Synechocystis* cultures kept in the light for 30 min, because under these conditions the areas above the fluorescence induction curve with DCMU (S_{DCMU}) were the same for all cell variants. Area ratio is approximately the same for wild-type and SDH$^-$ mutant, while mutant M55 ratio is much lower compared to the wild-type (Fig. 2).

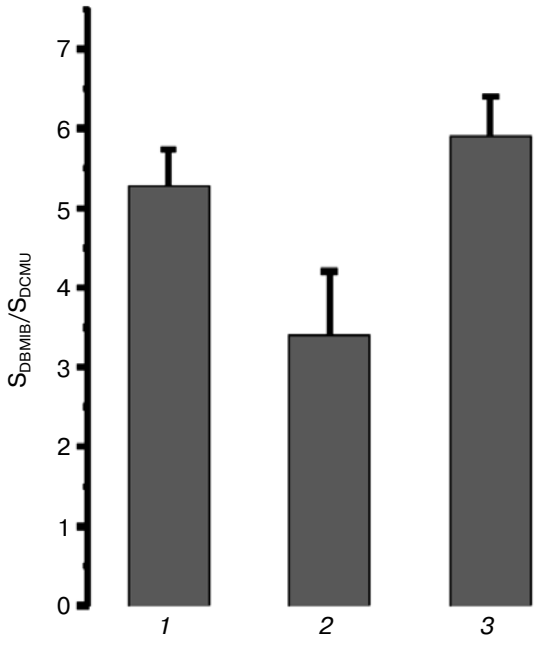


Fig. 2. Ratio of area above the fluorescence induction curve in the presence of 60 μM DBMIB (S_{DBMIB}) to the area above the fluorescence induction curve in the presence of 5·10^{-5} M DCMU and 60 μM DBMIB (S_{DCMU}), calculated for *Synechocystis* cells of wild-type (*1*) and mutants M55 (*2*) and SDH$^-$ (*3*), illuminated for 30 min with white light (25 μE·m^{-2}·sec^{-1}); ratios represent averages of five independent experiments.

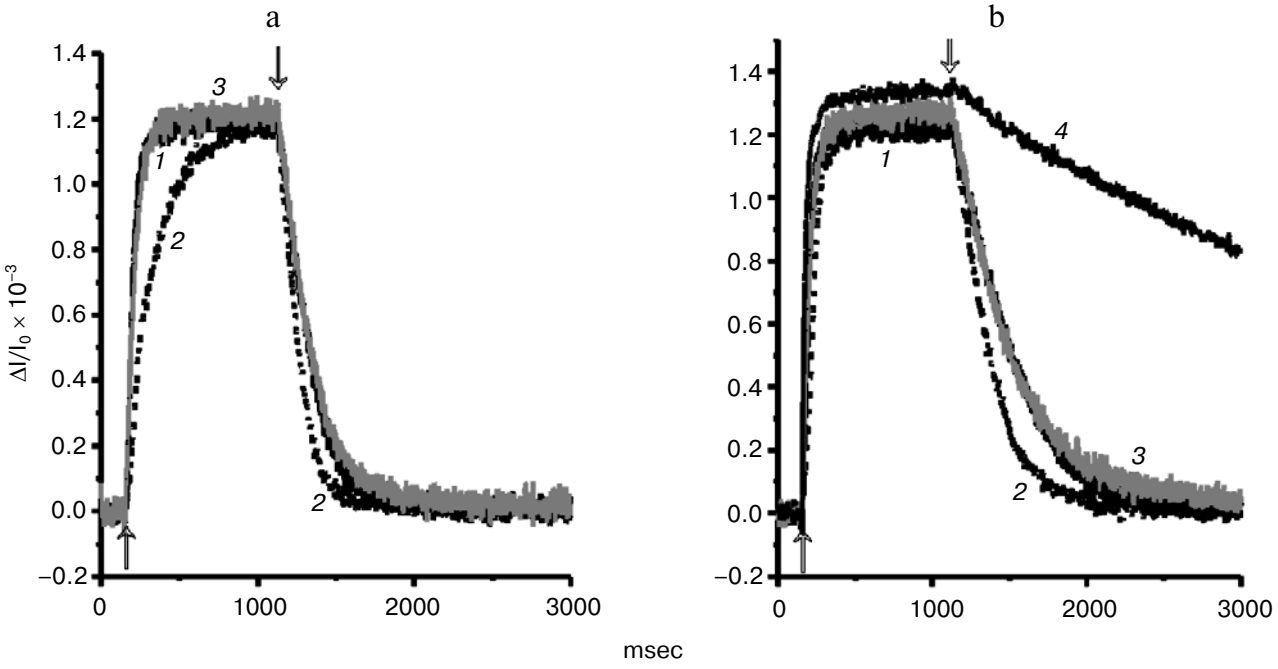


Fig. 3. Kinetics of photoinduced changes in absorption at 810 nm in *Synechocystis* cells of wild-type (*1*) and mutants M55 (*2*) and SDH$^-$ (*3*) in the presence of $5 \cdot 10^{-5}$ M DCMU, as well as of the wild type in the presence of $5 \cdot 10^{-5}$ M DCMU and 60 μM DBMIB (*4*), kept in the light for 30 min (a) or in the dark for 2 h (b) (730-nm actinic light, 4200 $\mu E \cdot m^{-2} \cdot sec^{-1}$ for 1 sec, the chlorophyll content in the sample was 5 μg/ml).

These data are hardly explained by more reduced PQ pool, since the PQ pool in the M55 mutant is more oxidized than in wild-type [25]. Since DBMIB is an oxidized analog of plastoquinones and affects only plastoquinol oxidase- but not plastoquinone reductase activity of the Q-cycle in low concentrations [28, 29], it is more likely that the lower areas ratio for M55 mutant compared to SDH$^-$ and wild-type is associated with an accelerated electron transfer from stroma to the PQ pool in the presence of DBMIB.

To test this hypothesis we studied P700 redox transformations in *Synechocystis* cells kept in the light or in darkness, during the cyclic electron transport with 730-nm actinic light. All measurements of P700 photooxidative reactions in this and subsequent experiments were performed in the presence of DCMU to eliminate the contribution of PSII to reduction of terminal electron acceptor $NADP^+$ on exposure to the actinic light. The rate of $P700^+$ dark reduction in preilluminated cells of wild type and mutants (Fig. 3a) was higher than in cells kept in darkness (Fig. 3b). Mutant M55 was observed to have the highest rate of $P700^+$ dark reduction both after illumination with white light for 30 min and after 2-h incubation of cells in darkness (see table), which may indicate a high rate of cyclic electron transport through the PSI in this mutant. Judging by the slow kinetics of P700 photooxidation in M55 cells, we can assume that the intensity of 730-nm actinic light (4200 $\mu E \cdot m^{-2} \cdot sec^{-1}$) is not sufficient for the maximum oxidation of P700. To verify this, we measured the light curves of P700 oxidation in wild-type and mutant *Synechocystis* cells. Indeed, $P700^+$ content increased with increasing intensity of 730-nm light significantly slower for M55 mutant cells than for wild-type cells and SDH$^-$ mutant cells, both after illumination with white light for 30 min and after 2 h of darkness (Fig. 4).

It was shown previously that the final acceptor pool ($NADP^+$) of the photosynthetic electron transport is almost completely reduced in M55 mutant due to changes in cell metabolism, whereas the pyridine nucleotide phosphate pool is reduced by only 70 and 50% in wild-type and SDH$^-$ mutant cells, respectively [25]. Thus, both the highest rate of $P700^+$ dark reduction and a somewhat slow oxidation of P700 in M55 cells in cyclic transport may be caused by the lack of terminal electron acceptor $NADP^+$, which results in faster transfer of electrons through reduced ferredoxin to plastoquinone (acceleration of the Fd-dependent cycle). The increase in the rate of $P700^+$ dark reduction, observed in all preilluminated cultures as compared to the cells kept in darkness for 2 h (see table), may also be caused by the lack of $NADP^+$ and higher levels of NADPH, since this reducing agent accumulates in the light in cells of photosynthetic organisms [17, 22]. Some increase in $\tau_{1/2}$ for the cells of the SDH$^-$ mutant compared with wild-type and M55 mutant cells (see table) may be associated with higher $NADP^+$ levels in stroma of SDH$^-$ mutant cells [25].

The rate of $P700^+$ dark reduction increased in all investigated cases after addition of the respiration

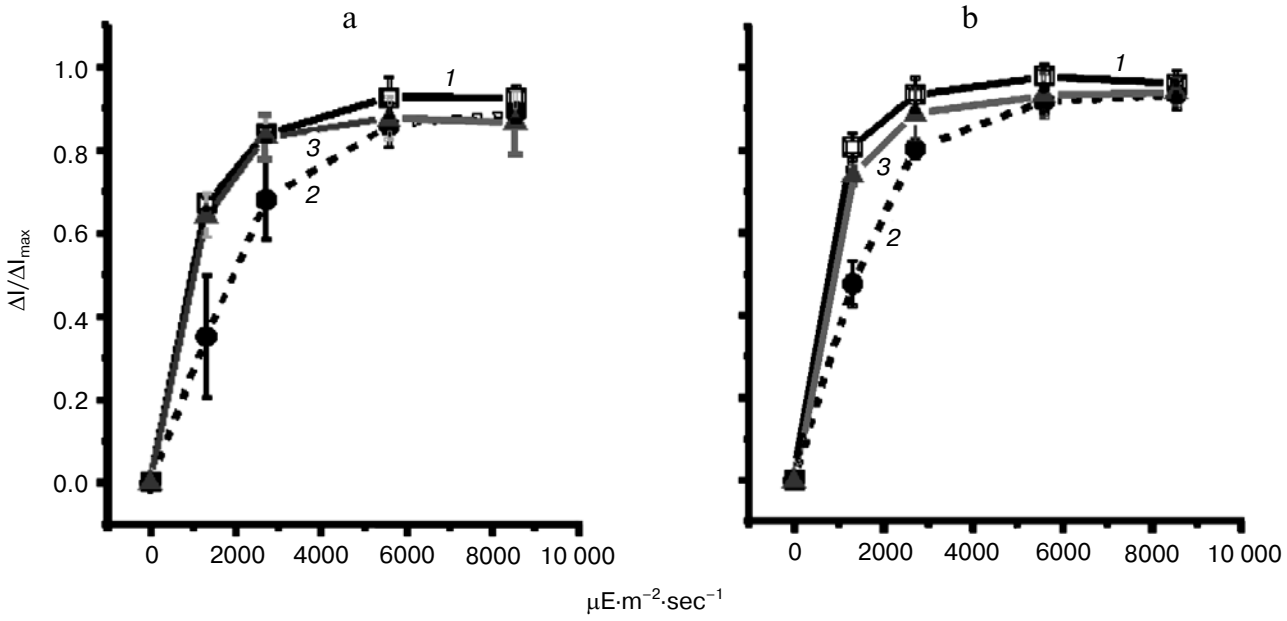


Fig. 4. Photoinduced changes in P700 absorption at 810 nm in *Synechocystis* cells of wild-type (*1*, square) and mutants lacking NDH-1 (*2*, black circle) or succinate dehydrogenase (*3*, triangle), kept in the light for 30 min (a) or in darkness for 2 h (b) in the presence of $5 \cdot 10^{-5}$ M DCMU. The curves are plotted from average values of five independent experiments; chlorophyll content 5-7 μg/ml; 730-nm actinic light, 1-sec duration.

inhibitor KCN and glucose to *Synechocystis* cells (Fig. 5 and the table). *Synechocystis* is a photoheterotrophic organism that can take up glucose and oxidize it in the pentose phosphate pathway reactions, creating reducing agent NADPH [14, 30], which supplies electrons to the PQ pool via dehydrogenase NDH-1 [11, 13, 14]. *Synechocystis* cells accumulate plastoquinols and NADH when incubated in the presence of KCN. In addition, cyanide inhibits ribulose-bisphosphate carboxylase activity in cells kept in the light [31], which leads to decrease in NADPH consumption in carbon cycle reactions. Therefore, $P700^+$ reduction rate in the presence of KCN and glucose in wild-type cells is expected to be the fastest, and $P700^+$ dark reduction must be slower in mutants lacking dehydrogenase than in the wild type. However, according to the table and Fig. 5, $P700^+$ dark reduction half-time is approximately the same for all the types of cells in the used conditions of light treatment, and the rate of $P700^+$ dark reduction is higher in cells that were preilluminated for 30 min than in cells kept in darkness.

Time of 50% dark reduction of $P700^+$ ($\tau_{1/2}$) calculated from the kinetics of photoinduced changes in absorption at 810 nm of *Synechocystis* cells: wild-type (WT) and mutants M55 or SDH^-

Treatment	$\tau_{1/2}$, msec		
	WT	M55	SDH^-
Light, 30 min	188 ± 46	131 ± 38	220 ± 70
Darkness, 2 h	415 ± 103	185 ± 42	416 ± 110
Light, 30 min +KCN +glucose	101 ± 21	107 ± 13	136 ± 33
Darkness, 2 h	177 ± 19	152 ± 20	205 ± 31

Note: Cells were kept in the light for 30 min or in darkness for 2 h in the presence of $5 \cdot 10^{-5}$ M DCMU or $5 \cdot 10^{-5}$ M DCMU, 3 mM KCN, and 10 mM glucose (730-nm actinic light, 4200 μE·m^{-2}·sec^{-1}, 1 sec); time values represent the average of five independent experiments.

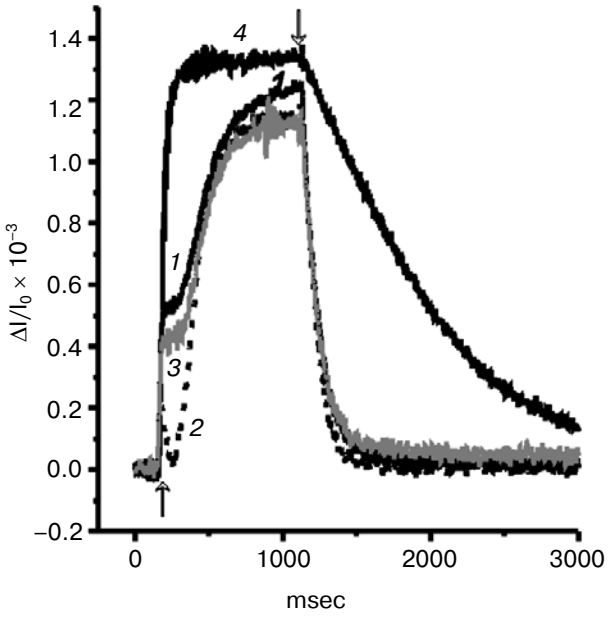


Fig. 5. Kinetics of photoinduced changes in absorption at 810 nm of *Synechocystis* cells, kept in the light for 30 min: wild type (*1*), mutants M55 (*2*) and SDH$^-$ (*3*) in the presence of $5 \cdot 10^{-5}$ M DCMU, 3 mM KCN, and 10 mM glucose, and mutant M55 in the presence of $5 \cdot 10^{-5}$ M DCMU, 3 mM KCN, 10 mM glucose, and 100 μM DBMIB (*4*); chlorophyll content 5 μg/ml, 730-nm actinic light (4200 μE·m^{-2}·sec^{-1}) and 1-sec duration.

Apparently, the rate of P700$^+$ dark reduction increases significantly after 30 min of illumination in lack of terminal electron acceptor NADP$^+$ and does not significantly depend on the presence of the dehydrogenases. We should note that the lowest rate of P700 photooxidation in the presence of KCN and glucose is observed in M55 mutant cells (Fig. 5, curve *2*), which may also indicate a lack of oxidized PSI electron acceptors. The rate of P700 photooxidation increases substantially in the presence of 100 μM DBMIB, and P700$^+$ dark reduction is slowed in cells of the wild type and the two mutants of *Synechocystis* treated with DCMU, KCN, and glucose (Fig. 5 represents the data for mutant M55 (curve *4*)). The findings indicate that cyclic transport through PSI goes through the Fd and PQ pools in the presence of excess reducing agent (in the presence of KCN and glucose) with little dependence on the presence of the dehydrogenases.

Slowing of dark reduction of PSI reaction centers in mutant M55, lacking dehydrogenase NDH-1, can still be detected, if cells are additionally illuminated immediately before the measurement with 680-nm red light with intensity of 1300 μE·m^{-2}·sec^{-1} for 1.5 min in the presence of DCMU (Fig. 6). Figure 6a shows a significant acceleration of P700 oxidation and slowing of P700$^+$ dark reduction in mutant M55 after additional illumination, but only after the first flash (730 nm, 4200 μE·m^{-2}·sec^{-1}, 0.5 sec) (curves *1* and *2*). P700 oxidation already slows

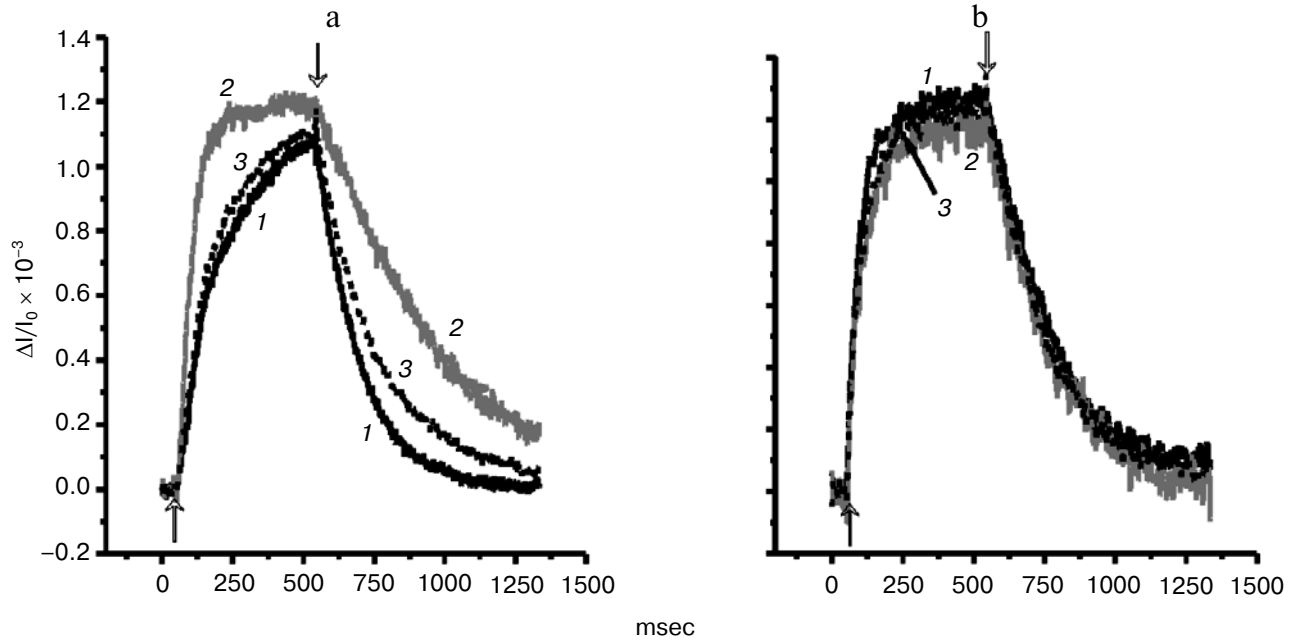


Fig. 6. Kinetics of photoinduced changes in absorption at 810 nm in *Synechocystis* mutant cells M55 (a) and SDH$^-$ (b) kept in the light for 30 min and preilluminated directly before the measurement with 680-nm light, 1300 μE·m^{-2}·sec^{-1} for 1.5 min in the presence of $5 \cdot 10^{-5}$ M DCMU. The following kinetics are shown: *1*) before the additional illumination with 680-nm light; *2*) immediately after illumination; *3*) after 14 sec of darkness; the chlorophyll content was 5 μg/ml; 730-nm actinic light (4200 μE·m^{-2}·sec^{-1}) and 0.5-sec duration.

and reduction accelerates after the second and all subsequent flashes of light, the interval between which was 14 sec (Fig. 6a, curve *3*). Such changes in the kinetics of P700 photoconversions are observed neither for wild type nor for SDH^- mutant (Fig. 6b). As M55 mutant is characterized by greater content of reduced terminal acceptor NADPH in stroma of cells than wild-type and SDH^- cells [25], it is possible that the excess NADPH in M55 cells triggers a reverse reaction of Fd reduction (with participation of FNR) and subsequent Fd-dependent reduction of the PQ pool during the period of darkness (14 sec) following the first flash. Then, we observe slowing of oxidation and the acceleration of $P700^+$ dark reduction already after the second flash. Thus, the Fd-dependent loop compensates for the absence of NDH-1 dehydrogenase in the mutant M55 cells, and the flash of 730-nm light for 1 sec (in the presence of DCMU) is not enough to detect slowing of $P700^+$ dark reduction caused by the absence of NDH-1 dehydrogenase.

In addition to $NADP^+$, oxygen is another final electron acceptor of PSI [32, 33]. Therefore, cyclic transport through PSI can be activated by lowering the level of final acceptors ($NADP^+$ and oxygen) in stroma of cells, given that *Synechocystis* cells are kept in conditions of $NAD(P)^+$ reduction (incubation of cells in the presence of KCN and glucose, see above) or reduced oxygen content (incubation of cells in glucose–glucose oxidase–catalase system). Figure 7 shows the kinetics of P700 redox reactions in *Synechocystis* wild-type cells (2 h in darkness), measured under conditions of changed donor/acceptor ratio in the stroma of the cells. The slowest oxidation of P700 and rapid reduction of $P700^+$ were observed in the glucose–glucose oxidase–catalase system in the presence of DCMU (Fig. 7, curve *1*). Under these conditions part of the glucose is taken up by the cells and then utilized in the first stage of the oxidative pentose phosphate pathway forming NADPH, and the rest is oxidized by added enzyme system while consuming the oxygen present in the medium in the sealed cell. This creates anaerobic conditions and blocks respiration. Faster kinetics of P700 oxidation and slower kinetics of $P700^+$ dark reduction are observed when keeping wild-type cells in the presence of KCN, glucose, and DCMU (Fig. 7, curve *2*). Glucose is also taken up under these conditions with co-formation of NADPH, and respiration is lacking, but the transfer of electrons from PSI to oxygen is not blocked and may even increase at low levels of $NADP^+$. Therefore, the respiratory inhibitor KCN does not affect cyclic electron transport through PSI as strongly as the anaerobic conditions. Even more rapid P700 oxidation and slower $P700^+$ reduction occur in the presence of DCMU alone with donor/acceptor ratio typical for the normal state of the cell stroma (Fig. 7, curve *3*). The most rapid oxidation and the slowest dark reduction of PSI reaction center is observed in the presence of DCMU and DBMIB, which inhibits the oxidation of the PQ pool,

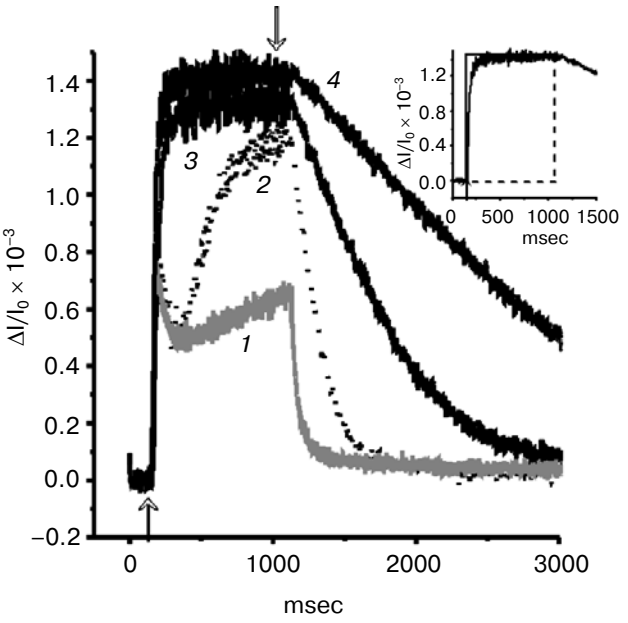


Fig. 7. Kinetics of photoinduced changes in absorption at 810 nm of *Synechocystis* wild-type cells, kept in darkness for 2 h: *1*) under anaerobic conditions (10 mM glucose, 24 units/ml glucose oxidase, and 400 units/ml catalase) plus $5 \cdot 10^{-5}$ M DCMU; *2*) in the presence of $5 \cdot 10^{-5}$ M DCMU, 3 mM KCN, and 10 mM glucose; *3*) in the presence of $5 \cdot 10^{-5}$ M DCMU; *4*) in the presence of $5 \cdot 10^{-5}$ M DCMU and 60 μM DBMIB. The inset shows the kinetics of P700 photoconversions in the presence of DCMU and DBMIB; marked lines limit the area (S_{max}) of theoretically maximum possible content of $P700^+$ under conditions practically eliminating the cyclic transport (see text). Chlorophyll content was 5 μg/ml, 730-nm actinic light (4200 $\mu E \cdot m^{-2} \cdot sec^{-1}$), and 1-sec duration.

preventing the transfer of electrons from stroma to the donor part of PSI (Fig. 7, curve *4*). Therefore, the less the content of PSI oxidized electron acceptors in the stroma is and the greater NADPH content is, the slower is P700 oxidation in the cyclic electron transport under 730-nm light, and the faster is dark reduction of $P700^+$.

Change in the absorption of plant cells at 810 nm versus 870 nm is due to the accumulation of oxidized plastocyanin and $P700^+$ on the donor side and reduced Fd in the acceptor side of PSI [34]. The change in absorption at 810 nm under conditions of low content of reduced Fd, for example, after far-red illumination, reflects the state of the donor side of PSI, which is close to a state of redox equilibrium between P700 and plastocyanin [34]. Apparently, the donor side of PSI in the presence of DCMU and DBMIB, when the maximum increase in absorption at 810 nm is observed, reflecting the maximum $P700^+$ content in the cells, is in a state close to equilibrium, i.e. electrons do not come into and are not removed from the equilibrium system plastocyanin–P700. It is obvious that high levels of reduced Fd accumulate under anaerobic conditions or in the presence of KCN and glucose, since under these conditions there is little oxidized

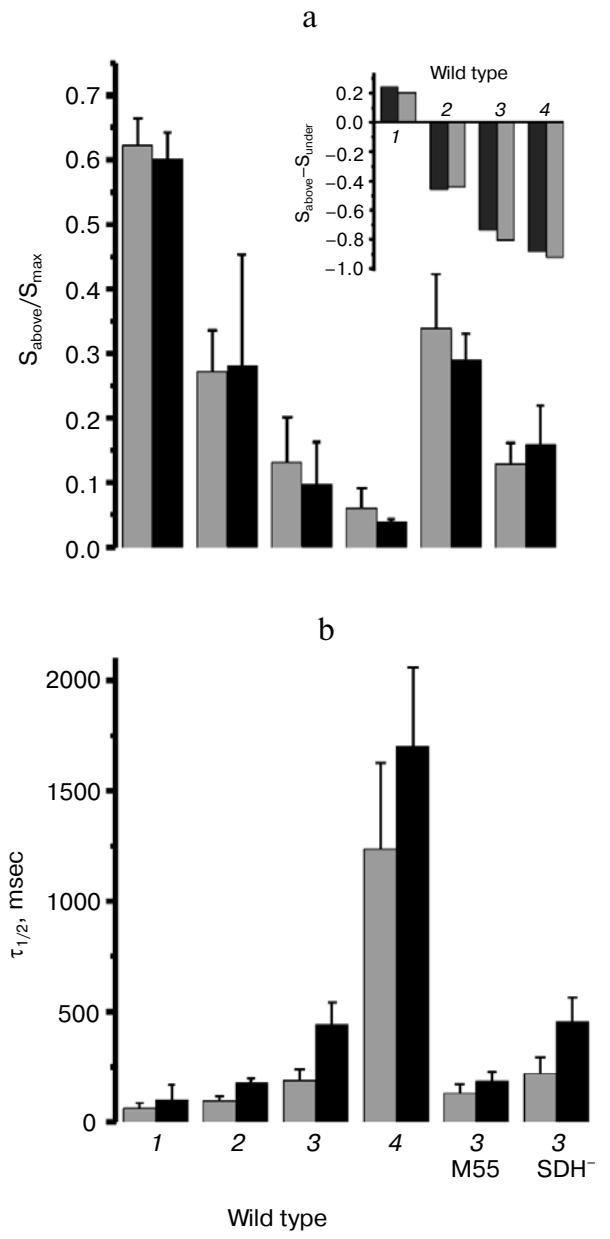


Fig. 8. a) Ratio of area above the curve of P700 photooxidation (S_{above}) for *Synechocystis* wild-type cells to the area limited by the theoretically maximal possible P700 oxidation line (S_{max}): *1*) under anaerobic conditions (+DCMU); *2*) in the presence of DCMU, KCN, and glucose; *3*) in the presence of DCMU; *4*) in the presence of DCMU and DBMIB; area ratio S_{above}/S_{max} for cells of M55 and SDH$^-$ mutants in the presence of DCMU. b) The average time of 50% dark reduction of P700$^+$ ($\tau_{1/2}$) for *Synechocystis* cells of wild-type and mutants M55 and SDH$^-$, measured under the same conditions as the area ratio. The inset shows the difference of areas above and below the photooxidation curve of P700 ($S_{above} - S_{under}$), calculated for the wild-type cells. Gray background, cells after 30 min of illumination; dark background, cells after 2 h of darkness. Concentrations of inhibitor, glucose, and enzymes for anaerobic conditions listed in the legend to Fig. 7. The diagrams are based on average values of five independent experiments.

PSI electron acceptors and, possibly, electron transport is uncoupled. Therefore, the lower the magnitude of the cell absorption change at 810 nm is and the greater the fraction of electrons transferred to the donor side of PSI during 1 sec of measurement with 730-nm actinic light is, the more electrons are at the acceptor side on Fd in the reduced state and the higher is the rate of P700$^+$ dark reduction. In this connection, it can be assumed that the area above the curve (S_{above}) is proportional to the fraction of electrons received by the donor side of PSI through PQ and Fd over 1 sec of illumination. The area below the curve (S_{under}) of P700 photooxidation is apparently proportional to the fraction of electrons transported through plastocyanin, P700, and Fd, including those that have reduced PQ in cyclic (mainly Fd-dependent) transport and re-passed through P700, and those that have not returned to the donor side of PSI as a result of oxygen or NADP$^+$ reduction. Then the difference of these areas will correspond to electrons reducing NADP$^+$ or oxygen, and we may estimate the content of available terminal PSI electron acceptor in the cells by this difference of areas. The total area above and below the curve of P700 photooxidation apparently reflects the maximal theoretically possible number of electrons that pass once through the donor side of PSI under conditions that prevent cyclic transport. This total area can be calculated theoretically if we accept that electron transport is practically nonexistent in the presence of DCMU and DBMIB. To calculate this area we drew a line at the level of maximum cell absorbance change at 810 nm in the presence of DCMU and DBMIB over 1 sec of illumination with 730-nm actinic light (Fig. 7, inset) and another line, denoting the most rapid oxidation of P700; then the area, reflecting a theoretical maximum content of P700$^+$, was calculated as the area of the resulting rectangle (Fig. 7, inset).

Figure 8a shows the areas above the curve of P700 photooxidation in wild-type cells of *Synechocystis* in conditions of altered donor/acceptor ratio in stroma, as well as the corresponding areas for the cells of M55 and SDH$^-$ mutants in the presence of DCMU. We should note that these areas represent the average ratios of the area above the P700 photooxidation curve to the area under the line, which limits the theoretical maximum possible oxidation of P700. Figure 8b shows the average time of 50% dark reduction of P700$^+$ ($\tau_{1/2}$) in *Synechocystis* wild-type cells under different conditions, which alter the donor/acceptor ratio in stroma, as well as the corresponding values for the cells of M55 and SDH$^-$ mutants in the presence of DCMU. Figure 8 display a clear correspondence between the area above the kinetic curve of P700 photooxidation over 1 sec with 730-nm actinic light and the time of 50% dark reduction of P700$^+$: the larger is the area, the higher is the rate of P700$^+$ reduction. It should be noted that the area above the curve and the time of the 50% dark reduction of P700$^+$ for the M55 mutant in the presence of DCMU is close to the corresponding value for the wild

type in the presence of KCN and glucose (+DCMU). This fact may indicate approximately the same level of acceptor $NADP^+$ in the stroma of these cells, since both M55 mutant [25] and wild type in the presence of KCN and glucose are characterized by low level of respiration and CO_2 fixation. These numbers for the cells of SDH^- mutant and wild type in the presence of DCMU are also roughly the same, which indicates similar levels of acceptor $NADP^+$ in the stroma of these cells.

The data indicate that the kinetics of P700 redox reactions in *Synechocystis* cells under 730-nm light in the presence of DCMU depends on the level of $NADP^+$ and oxygen in the stroma of the cells. The absence of respiratory dehydrogenases indirectly affects the rate of cyclic electron transport through PSI, apparently changing the donor/acceptor concentration ratio in the stroma, which regulates the rate of the Fd-dependent cycle.

DISCUSSION

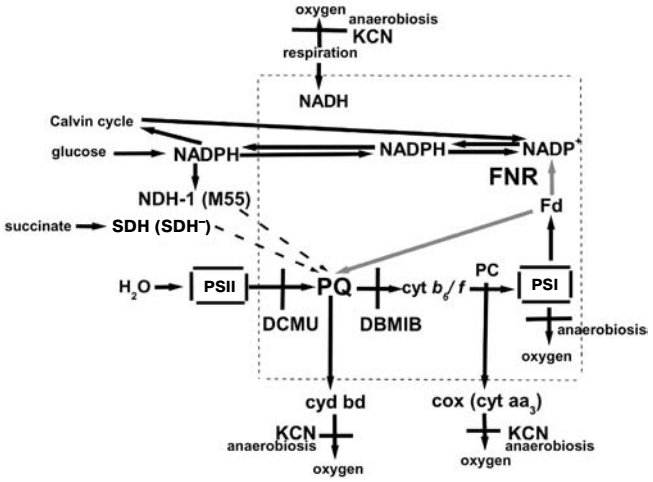
Removal of terminal PSI electron acceptor, oxygen, from the suspension of cyanobacteria cells (*Synechocystis* sp. or *Arthrospira platensis*) was previously shown to cause severe slowing of P700 oxidation, decreasing the level of $P700^+$ and accelerating its dark reduction in the presence of DCMU [32, 35], while increase of $NADP^+$ content during light activation of Calvin cycle reactions, on the contrary, results in acceleration of P700 photooxidation and deceleration of $P700^+$ dark reduction under anaerobiosis even in the presence of DCMU [35]. These data indicate the dependence of cyclic electron transport through PSI on redox state of PSI electron acceptor pools in stroma.

The findings also indicate that rate of cyclic electron transport through PSI depends on the electron acceptor levels ($NADP^+$ and oxygen) in all the variants of *Synechocystis* cells when registering the kinetics of P700 photoconversion with 730-nm actinic light (4200 $\mu E \cdot m^{-2} \cdot sec^{-1}$) with 1-sec duration. Kinetics of P700 photoconversions, measured under these conditions, does not reflect the lack of respiratory dehydrogenases in the mutant cells, and the cyclic transport of electrons into the donor part of PSI is carried out by an Fd-dependent pathway.

As M55 mutant of *Synechocystis* was characterized by the lowest level of $NADP^+$ acceptor compared with wild-type and SDH^- [25], it seems that it is precisely this fact that explains the highest rate of $P700^+$ dark reduction in cyclic electron transport through PSI (Fig. 3 and the table). The slow rate of $P700^+$ reduction in the mutant devoid of succinate dehydrogenase (SDH^-) can be attributed to high levels of $NADP^+$ and low content of succinate in these cells [25]. Slowing of P700 oxidation in the light and acceleration of $P700^+$ dark reduction can be caused not only by decrease in the level of terminal PSI electron acceptor in the environment (oxygen), but also by the action of an uncoupler [32]. It is very likely that the donor/acceptor concentration ratio in stroma alters the electrochemical potential on the thylakoid membrane by regulating the rate of cyclic electron flow through the PSI, which in turn regulates the rate of the Fd-dependent cycle.

The rate of $P700^+$ dark reduction in *Synechocystis* cells is significantly slower in M55 mutant as compared with wild-type after illumination with actinic light (>710 nm) for 45 sec [11]. We also detected slowing of $P700^+$ dark reduction in M55 mutant after additional illumination of cells with 680-nm light, 1300 $\mu E \cdot m^{-2} \cdot sec^{-1}$ for 1.5 min in the presence of DCMU (Fig. 6). In this study, 730-nm actinic light (1 sec) from an LED lamp was used for registration of the kinetics of P700 photoconversion, the light being absorbed only by the chlorophyll molecules of PSI, whereas 680-nm light is absorbed both by chlorophylls and phycobilisomes and should therefore be more efficient to induce photosynthetic electron transport. Therefore, we can assume that the additional illumination of M55 mutant cells with 680-nm light in the presence of DCMU mainly reduced not $NADP^+$, the content of which is extremely low [25], but oxygen, and electrons are removed from cyclic transport. This leads to an oxidation of the PQ pool and possibly of the Fd pool, which is detected by acceleration of P700 photooxidation and slowing of $P700^+$ dark reduction during the kinetics registration under 730-nm light after additional illumination (Fig. 6a). Since the reduction of oxygen by PSI, in particular, by ferredoxin, depends on the content of $NADP^+$ [33], it is likely that not oxygen, but preferably $NADP^+$ is reduced in the wild type and SDH^- mutant cells containing more $NADP^+$ acceptor as compared with M55 [25], and then the electrons from NADPH return to the PQ pool via Fd-dependent and partially NDH-1-dependent pathways [8-11] at higher rates than in the cells of the M55 mutant. This is indicated by the absence of differences in the kinetics of P700 photoconversions before and after additional illumination with 680-nm light between the cells of wild type and SDH^- mutant (Fig. 6b).

The difference of areas ($S_{above} - S_{under}$) above and below the kinetics curve of P700 photooxidation (Fig. 8a, inset) may represent the fraction of electrons that are not returned in the cycle because of DBMIB, due to oxygen reduction or NADPH consumption in the carbon cycle reactions. Therefore, the difference of areas has a negative value in case of DBMIB treatment (+DCMU), in the presence of DCMU alone, and even in the presence of DCMU, cyanide, and glucose. Thus, the more electrons do not return to the cycle, the less is the value of the area difference, and the slower is the dark reduction. Under anaerobic conditions (with DCMU) the area difference has a positive value, since under these conditions electrons do not leave the cycle, but, on the contrary, enter the cycle, perhaps due to a reverse ferredoxin reduction



Paths of electron in cyclic transport through the PSI. The dotted line encircled the reactions that presumably determine the kinetics of P700 redox transformations when measured in the presence of DCMU during 1-sec actinic illumination with 730-nm light. Dotted arrows indicate the reactions of plastoquinone reduction that are impaired in the mutant cells. The sites of action of photosynthesis inhibitors (DCMU and DBMIB) and terminal oxidase inhibitor (KCN) are shown; electron transfer to oxygen (including that through the PSI and in the process of respiration) is blocked by anaerobiosis; cyt b_6/f, cytochrome b_6/f complex. Gray arrows indicate the competing pathways of oxidation of reduced Fd, which depend on the $NADP^+$ and oxygen content in the cells

reaction with Fd-$NADP^+$-reductase with an excess reduced terminal acceptor NADPH, and significantly accelerate all reactions through PSI.

Thus, the data indicate that the kinetics of redox reactions of P700 in the cells of *Synechocystis*, measured during 1 sec of 730-nm actinic light in the presence of DCMU, mainly reflect the cyclic flow of electrons through PSI from PQ pool to Fd and back to the PQ pool. Reduction of the PQ pool in the Fd-dependent cycle competes with the reactions of NADPH consumption for electrons from Fd, and the level of reduced Fd depends on the availability of terminal PSI electron acceptors – $NADP^+$ and oxygen (see Scheme). Therefore, the kinetics of redox transformations of P700 in cells of *Synechocystis* under short-term illumination with 730-nm light in the presence of DCMU allow us to estimate the levels of terminal PSI acceptors and the donor/acceptor ratio in the stroma of the cells, i.e. the physiological state of wild-type or mutant cell cultures.

We thank Prof. T. Ogawa for kindly provided mutant *Synechocystis* sp. PCC 6803 M55 and Prof. W. F. J. Vermaas for the SDH^- mutant.

This study was supported by the Russian Academy of Sciences Presidium program "Molecular and Cell Biology" and Russian Foundation for Basic Research grants (08-04-00143a, 09-04-01119a, and 11-04-00247a).

REFERENCES

1. Howitt, C. A., Smith, G. D., and Day, D. A. (1993) *Biochim. Biophys. Acta*, **1141**, 313-320.
2. Sturzl, E., Scherer, S., and Boeger, P. (1984) *Physiol. Plant.*, **60**, 479-483.
3. Schmetterer, G. (1994) in *The Molecular Biology of Cyanobacteria* (Bryant, D. A., ed.) Kluwer Academic Publishers, Dordrecht, pp. 409-435.
4. Cooley, J. W., Howitt, C. A., and Vermaas, W. F. J. (2000) *J. Bacteriol.*, **182**, 714-722.
5. Schultze, M., Forberich, B., Rexroth, S., Gwendolyn Dyczmons, N., Roegner, M., and Appel, J. (2009) *Biochim. Biophys. Acta*, **1787**, 1479-1485.
6. Maxwell, P. C., and Biggins, J. (1976) *Biochemistry*, **15**, 3975-3981.
7. Joliot, P., and Joliot, A. (2006) in *Photosynthesis and Respiration*, Vol. 24 (Golbeck, J. H., ed.) Springer, Dordrecht, pp. 639-656.
8. Jeanjean, R., van Thor, J. J., Havaux, M., Joset, F., and Matthijs, H. C. P. (1999) in *The Phototrophic Prokaryotes* (Peschek, G. A., Loffelhardt, W., and Schmetterer, G., eds.) Kluwer/Plenum, New York, pp. 251-258.
9. Shikanai, T. (2007) *Annu. Rev. Plant Biol.*, **58**, 199-217.
10. Yeremenko, N., Jeanjean, R., Prommeenate, P., Krasikov, V., Nixon, P. J., Vermaas, P. J. W., Havaux, M., and Matthijs, H. C. P. (2005) *Plant Cell Physiol.*, **46**, 1433-1436.
11. Mi, H., Endo, T., Schreiber, U., Ogawa, T., and Asada, K. (1992) *Plant Cell Physiol.*, **33**, 1233-1237.
12. Mi, H. L., Klughammer, C., and Schreiber, U. (2000) *Plant Cell Physiol.*, **41**, 1129-1135.
13. Mi, H., Endo, T., Schreiber, U., Ogawa, T., and Asada, K. (1994) *Plant Cell Physiol.*, **35**, 163-173.
14. Elanskaya, I. V., Timofeev, K. N., Grivennikova, V. G., Kuznetsova, G. V., Davletshina, L. N., Lukashev, E. P., and Yaminsky, F. V. (2004) *Biochemistry (Moscow)*, **69**, 445-454.
15. Gotoh, E., Matsumoto, M., Ogawa, K., Kobayashi, Y., and Tsuyama, M. (2010) *Photosynth. Res.*, DOI: 10.1007/s11120-009-9525-0.
16. Oja, V., Eichelmann, H., Peterson, R. B., Rasulov, B., and Laisk, A. (2003) *Photosynth. Res.*, **78**, 1-15.
17. Endo, T., Kawase, D., and Sato, F. (2005) *Plant Cell Physiol.*, **46**, 775-781.
18. Hald, S., Nandha, B., Gallois, P., and Johnson, G. N. (2008) *Biochim. Biophys. Acta*, **1777**, 433-440.
19. Ogawa, T. (1991) *Proc. Natl. Acad. Sci. USA*, **88**, 4275-4279.
20. Rippka, R., Deruelles, Waterbury, J. B., Herdman, M., and Stanier, R. Y. (1979) *J. Gen. Microbiol.*, **111**, 1-61.
21. Lichtenthaler, H. K. (1987) in *Methods in Enzymology*, Vol. 148 (Colowick, S. P., and Kaplan, N. O., eds.) Academic Press Inc., San Diego, pp. 350-382.
22. Tamoi, M., Miyazaki, T., Fukamizo, T., and Shigeoka, S. (2005) *The Plant J.*, **42**, 504-513.
23. Schreiber, U., Schliwa, U., and Bilger, W. (1986) *Photosynth. Res.*, **10**, 51-62.
24. Schreiber, U., Klughammer, C., and Neubauer, C. (1988) *Z. Naturforsch.*, **43c**, 686-698.
25. Cooley, J. W., and Vermaas, W. F. J. (2001) *J. Bacteriol.*, **183**, 4251-4258.

26. Berry, S., Schneider, D., Vermaas, W. F. J., and Roegner, M. (2002) *Biochemistry*, **41**, 3422-3429.
27. Mullineaux, C. W., and Allen, J. F. (1988) *Biochim. Biophys. Acta*, **934**, 96-107.
28. Nanba, M., and Katoh, S. (1984) *Biochim. Biophys. Acta*, **767**, 396-403.
29. Jones, R. W., and Whitmarsh, J. (1988) *Biochim. Biophys. Acta*, **933**, 258-268.
30. Moezelaar, R., and Stal, L. J. (1994) *Arch. Microbiol.*, **162**, 63-69.
31. Kobayashi, Y., and Heber, U. (1994) *Photosynth. Res.*, **41**, 419-428.
32. Trubitsin, B. V., Ptushenko, V. V., Koksharova, O. A., Mamedov, M. D., Vitukhnovskaya, L. A., Grigor'ev, I. A., Semenov, A. Yu., and Tikhonov, A. N. (2005) *Biochim. Biophys. Acta*, **1708**, 238-249.
33. Kozuleva, M. A., and Ivanov, B. N. (2010) *Photosynth. Res.*, **105**, 51-61.
34. Oja, V., Eichelmann, H., Anijalg, A., Raemma, H., and Laisk, A. (2010) *Photosynth. Res.*, **103**, 153-166.
35. Bolychevtseva, Yu. V., Terekhova, I. V., Roegner, M., and Karapetyan, N. V. (2007) *Biochemistry (Moscow)*, **72**, 275-281.