Biochemistry (Moscow), Vol. 70, No. 12, 2005, pp. 1390-1395. Translated from Biokhimiya, Vol. 70, No. 12, 2005, pp. 1688-1695.

Effects of Dark Adaptation on Light-Induced Electron Transport through Photosystem I in the Cyanobacterium *Synechocystis* sp. PCC 6803

K. N. Timofeev[1], G. V. Kuznetsova[1], and I. V. Elanskaya[2]*

[1]Department of Biophysics and [2]Department of Genetics, Faculty of Biology, Lomonosov Moscow State University, 119992 Moscow, Russia; fax: (7-095) 939-2957; E-mail: ivelanskaya@mail.ru

Received December 16, 2004
Revision received March 14, 2005

Abstract—Kinetics of the redox reactions in the reaction center (P700) of photosystem I (PSI) of the cyanobacterium *Synechocystis* sp. PCC 6803 have been studied by EPR spectroscopy. The redox kinetics were recorded based on accumulation of the EPRI signal when the final signal was the sum of individual signals produced in response to illumination of the cells. After prolonged (more than 3 sec) dark intervals between illuminations, the kinetic curve of the EPR signal from $P700^+$ was multiphasic. After a sharp increase in the signal amplitude at the beginning of illumination (phase I), the amplitude rapidly (for 0.1-0.2 sec) decreased (phase II). Then the signal amplitude gradually increased (phase III) until the steady rate of electron transfer was established. With short-term (1 sec) dark intervals between the flashes and also in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), the kinetics of the light-induced increase in the EPR signal from $P700^+$ were monophasic. Inhibition with iodoacetamide of electron transport on the acceptor side of PSI under anaerobic conditions or an increase in the amount of respiration substrates on addition of glucose into a suspension of DCMU-treated wild-type cells increased the level of P700 reduction in phase III. The findings suggest that the kinetic curve of the EPR signal from $P700^+$ is determined by both the electron entrance onto $P700^+$ on the donor side of PSI and activity of electron acceptors of PSI.

Key words: cyanobacterium *Synechocystis* 6803, electron transport, photosystem I, EPR spectroscopy

Unicellular photoheterotrophic cyanobacteria, such as *Synechocystis* sp. PCC 6803 (further *Synechocystis* 6803), can grow both photoautotrophically via oxygenic photosynthesis and heterotrophically with glucose as a source of carbon [1, 2]. Under photoautotrophic conditions, these organisms fix CO_2 in the Calvin cycle [3], while during heterotrophic metabolism glucose is assimilated via the oxidative pentose phosphate cycle [4]. Fixation of CO_2 is suppressed in the dark and G6P dehydrogenase, which is a key enzyme of glucose assimilation in the oxidative pentose phosphate cycle, is activated [3]. The activity of G6P dehydrogenase is suppressed in the light with ribulose diphosphate (RuDP) generated in the Calvin cycle. And phosphoribulokinase, which catalyzes the formation of RuDP, is induced by light and rapidly inactivated in the dark [1, 3-6].

In the thylakoid membrane of *Synechocystis* 6803 both photosynthetic and respiratory electron transport occurs [7]. The plastoquinone (PQ) pool, cytochrome b_6f complex, and a soluble cytochrome c_{553} (or plastocyanine) are common components of the photosynthetic and respiratory electron transport chains (ETC) [8]. In the light electrons enter the PQ pool from photosystem II (PSII) and are transmitted onto the light-oxidized reaction center of PSI ($P700^+$). NADPH produced in the cyanobacterium cells in the light as a result of the noncyclic electron transport through PSI is oxidized in the course of various reactions including light-induced fixation of CO_2, cyclic electron transport with involvement of PSI [9, 10], and reduction of oxygen to water with involvement of flavoproteins [11]. In the dark NADPH is produced during oxidation of glucose (or endogenous glycogen), and the PQ pool is reduced due to entrance of electrons from respiratory dehydrogenases (NDH-1, NDH-2), succinate dehydrogenase (SDH), and oxidized under the influence of terminal cytochrome and quinol oxidases [12-15]. Thus, activities of the photosynthetic and respiratory ETC components are regu-

Abbreviations: PS) photosystem; ETC) electron transport chain; DCMU) 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Fd) ferredoxin; G6P) glucose-6-phosphate; IAC) iodoacetamide; PQ) plastoquinone; RuDP) ribulose-1,5-diphosphate.

* To whom correspondence should be addressed.

0006-2979/05/7012-1390

lated on the level of the PQ pool and the cytochrome b_6f complex.

EPR spectroscopy is useful for studies on the interaction between the photosynthetic and respiratory ETCs because it allows us to study electron transport through PSI in intact cyanobacterial cells. We have earlier shown that the rate of reduction of $P700^+$ oxidized in response to a white light pulse in photoautotrophically grown *Synechocystis* 6803 cells depends on the entrance onto PQ of electrons from PSII, exogenous glucose, and reduced quinones [16, 17]. In these works, the accumulation of the EPR signal from $P700^+$ was used when the final signal was the sum of individual signals obtained in response to illuminations of cells alternating with dark intervals of certain duration. Because the PQ pool, which is a donor of electrons for the light-oxidized $P700^+$, can be reduced in the dark by electrons from respiration substrates, in the present work we have studied the influence of dark adaptation of the cells on the light-induced electron transport through PSI.

MATERIALS AND METHODS

Strains and culture conditions. Wild-type cells of the cyanobacterium *Synechocystis* sp. PCC 6803 were grown photoautotrophically for three days at 30°C with uninterrupted illumination of 50 $\mu E \cdot m^{-2} \cdot sec^{-1}$ in liquid mineral medium BG-11 [2].

EPR spectroscopy. The cells were precipitated by centrifugation and suspended in BG-11 medium containing 10 mM Hepes-NaOH (pH 7.5). The cell suspension with the chlorophyll concentration of about 30 μg/ml was preincubated in the dark for 20 min and then placed in a quartz cuvette. The EPRI signal was induced with white light (3000 $\mu E \cdot m^{-2} \cdot sec^{-1}$). Redox kinetics of P700 was recorded at room temperature with a PE-1304 EPR spectrometer of the 3-cm range (Chernogolovka, Russia). The magnetic field was fixed on the high-field peak of the EPRI signal derivative. The signal from $P700^+$ was recorded with signal averaging, the final signal being a sum of 20 individual signals obtained in response to separate illuminations. Previously to recording the EPR signal, the cuvette with the suspension placed into the EPR spectrometer was illuminated 10 times with a white light alternating with dark intervals of a definite duration to adapt the cells to conditions of the experiment. Durations of the light and dark periods were determined by the experiment conditions.

The EPR conditions were as follows: UHF power 20 mW, modulation amplitude 0.3 mT, modulation frequency 100 kHz, time constant 10 μsec. DCMU (20 μM), iodoacetamide (IAC) (8 mM), and glucose (5 mM) were added into the cell suspension immediately before recording EPR spectra. Anaerobic conditions were created by blowing argon through the cell suspension.

RESULTS

Dependence of kinetics of redox transformations of P700 in *Synechocystis* 6803 cells on duration of dark adaptation periods. The light-induced EPRI signal appears on the light-induced oxidation of the reaction centers of PSI (P700). On illumination of the wild-type *Synechocystis* 6803 cells with white light after short (1 sec) dark intervals, the amplitude of the signal from $P700^+$ sharply increased immediately after the start of illumination, then for about 0.5 sec achieved a steady level which retained until the light was switched off (Fig. 1a). On prolonging the periods of dark adaptation the kinetic curve of the EPR signal from $P700^+$ displayed three distinct phases: (I) a rapid (less than 0.1 sec) increase in the amplitude of the EPR signal corresponding to the light-induced oxidation of P700; (II) a rapid (0.1-0.2 sec) reduction of $P700^+$ in the light; and (III) a gradual increase in the amplitude of the signal from $P700^+$ until a steady state of the electron transfer was attained (Fig. 1, b-d).

The decrease in the amplitude of the EPR signal from $P700^+$ in phase II deepened with increase in duration of dark intervals between the cell illuminations, suggesting the increased reduction of P700 in the light. It seemed that the dark adaptation of the cells was associated with accumulation of reductants on the donor side of PSI, and these reductants markedly influenced the kinetics of light-induced redox reactions in P700.

In the light the PQ pool, which is the main electron donor for $P700^+$, was reduced chiefly by electrons from PSII. To reveal the effects of reductants accumulated during the dark adaptation of the cells on the light-induced electron transport through PSI, the redox reaction of P700 was studied in the presence of DCMU, which inhibited the electron flow from PSII, and also on addition of glucose as a source of respiration substrates in the dark.

Effects of DCMU and glucose on kinetics of redox transformations of P700 in *Synechocystis* 6803 cells. In the presence of DCMU, the kinetic curve of the signal from

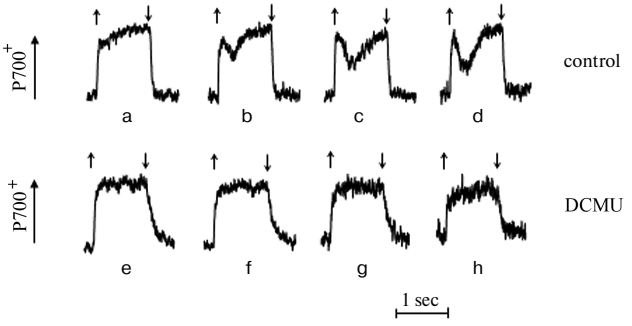

Fig. 1. Kinetics of redox transformations of P700 in *Synechocystis* 6803 cells illuminated with white light for 1 sec in the absence of DCMU (a-d) and in the presence of 20 μM DCMU (e-h). The duration of the dark interval between the cell illuminations was 1 (a, e), 3 (b, f), 5 (c, g), and 10 sec (d, h). The arrows indicate time of switching the light on (↑) and off (↓).

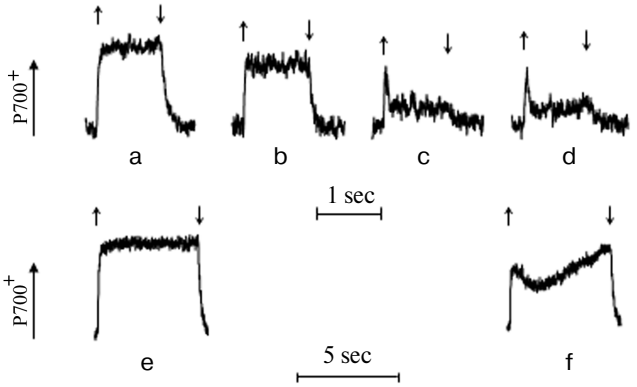

Fig. 2. Kinetics of redox transformations of P700 in *Synechocystis* 6803 cells in the presence of 20 μM DCMU and 5 mM glucose on illumination with white light for 1 (a-d) and 5 sec (e, f). The duration of dark intervals between the cell illuminations was 1 (a, e), 3 (b), 5 (c), and 10 sec (d, f).

$P700^+$ in the *Synechocystis* 6803 cells was monophasic independently of duration of the light and dark periods. The amplitude of the signal from $P700^+$ sharply increased after the beginning of illumination and reached at once a steady state, which was maintained until the light was switched off (Fig. 1, e-h). However, with increase in the duration of the dark adaptation periods the amplitude of the EPRI signal gradually decreased, suggesting a slight increase in the level of P700 reduction.

The addition of 5 mM glucose to a suspension of DCMU-treated cells did not change the kinetic curve of the signal from $P700^+$ at the dark intervals of 1 sec (Fig. 2a). Prolongation of dark periods to 3 sec resulted in a decrease in the amplitude of the signal from $P700^+$, but had no influence on the monophasic character of the kinetic curve (Fig. 2b). With more prolonged dark intervals (5-10 sec), the level of P700 reduction in the light significantly increased on the addition of glucose, which was displayed by considerably decreased amplitude of the signal from $P700^+$ in phase III. This was associated with appearance of distinct phase II in the kinetic curves, and after this phase P700 remained in a partially reduced state for 1-2 sec (Fig. 2, c and d). During the more prolonged illuminations of the cells in the presence of glucose, the amplitude of the signal from $P700^+$ gradually increased until a steady oxidized state was attained (Fig. 2f).

The presented data show that the kinetic curve of the light-induced changes in the EPR signal from $P700^+$ depends on both the activity of the noncyclic photosynthetic electron transport and the presence of cytoplasmic respiration substrates. The effect of exogenous glucose was observed only in the case of prolonged dark intervals between the cell illuminations, and this was likely to be associated with the activation of a dark current.

Dependence of post-illumination reduction of $P700^+$ in the presence of DCMU and glucose on duration of light and dark intervals. After switching off the light, the photooxidized $P700^+$ in *Synechocystis* 6803 cells is reduced due to entrance of electrons from the plastoquinone pool through the cytochrome b_6f complex [17]. Figure 3 presents kinetic curves of redox transformations of P700 on illumination with short-term (0.05 sec) light flashes with dark intervals of 1 sec. In the absence of DCMU the photooxidized $P700^+$ rapidly reduced after the light was switched off (Fig. 3a). Addition of DCMU suppressed the electron transfer from PSII and noticeably decreased the rate of the $P700^+$ signal decay (Fig. 3b). In the presence of 5 mM glucose, the rate of $P700^+$ reduction in DCMU treated cells after the light was switched off increased again (Fig. 3c). Thus, after the short-term illumination of the cells in the presence of DCMU electrons of the respiration substrates generated during oxidation of exogenous glucose entered onto the photooxidized $P700^+$ even with short periods of dark adaptation.

The rate of post-illumination reduction of $P700^+$ in the presence of DCMU significantly depended on duration of the periods of illumination and dark adaptation of the cells (Fig. 4a). With short illuminations (0.05-0.2 sec) in the presence of DCMU the rate of the $P700^+$ signal decay rapidly increased with increase in the duration of dark adaptation periods and at dark intervals of 5 sec and more was the same in both the presence and absence of DCMU (Fig. 4a). If the cells were illuminated for 0.5 sec or more, the rate of the post-illumination reduction of $P700^+$ pronouncedly decreased under the influence of DCMU under both short-term and prolonged dark intervals (Fig. 4a). Thus, in the case of prolonged illumination of the cells the PQ pool, which is a source of electrons for the photooxidized $P700^+$, seemed to be mainly reduced by electrons from PSII.

Addition of glucose to DCMU-pretreated cells increased the rate of the $P700^+$ signal decay after the light was switched off. Under short-term illumination (0.05 sec) the rate of the post-illumination reduction of $P700^+$ in the presence of glucose and DCMU reached the values characteristic for the DCMU-untreated cells at any duration of the dark intervals (Fig. 4b, curve *4*). Thus, in the

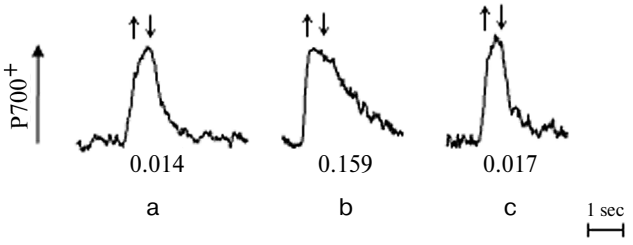

Fig. 3. Kinetics of redox transformations of P700 in *Synechocystis* 6803 cells illuminated with white light for 0.05 sec with dark intervals of 0.2 sec: a) control without DCMU and glucose; b) in the presence of 20 μM DCMU; c) in the presence of 20 μM DCMU and 5 mM glucose. The numbers under the curves indicate the half-time of the $P700^+$ signal decay ($t_{1/2}$, sec).

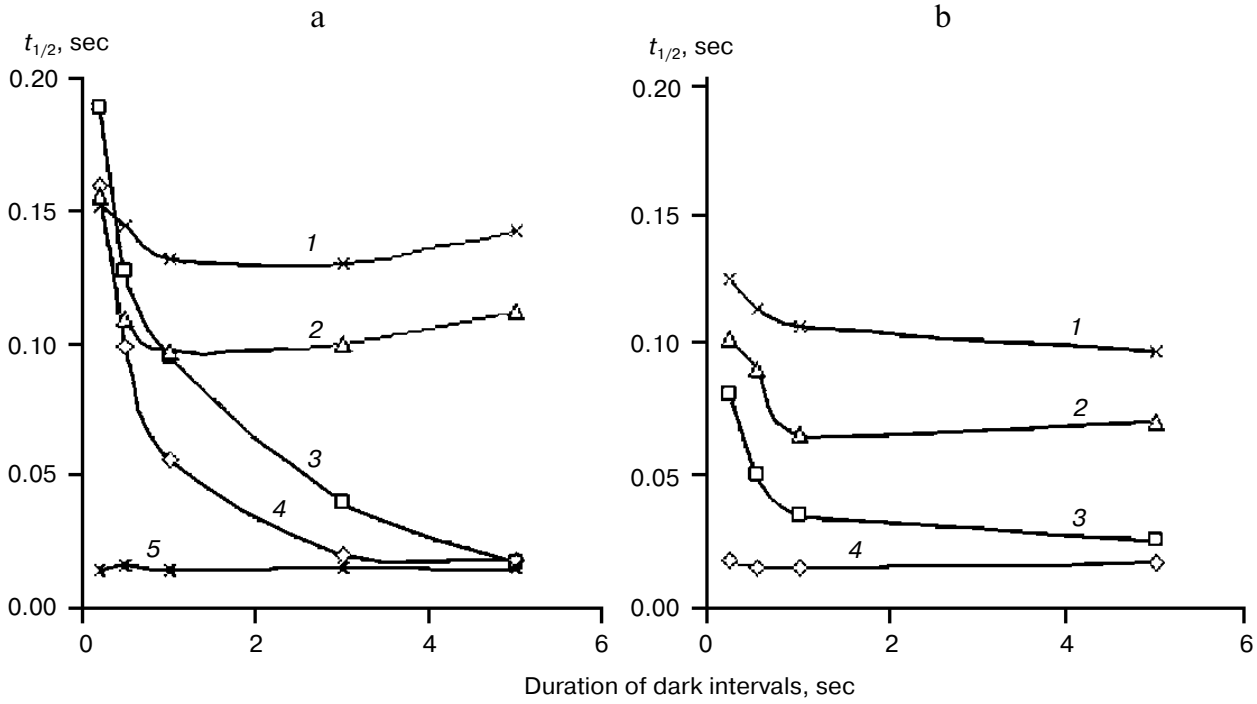

Fig. 4. Effect of DCMU on the rate of post-illumination reduction of $P700^+$ ($t_{1/2}$, sec) in the absence (a) and in the presence of 5 mM glucose (b) depending on duration of the light and dark intervals. The duration of the cell illumination with white light in the presence of DCMU was 1 (*1*), 0.5 (*2*), 0.2 (*3*), and 0.05 sec (*4*) with the dark intervals of varied duration. *5*) Control without DCMU regardless of the illumination duration.

presence of DCMU, electrons entering the PQ pool from exogenous glucose were involved in the reduction of the photooxidized $P700^+$. With increase in the duration of illumination periods, the effect of glucose was less pronounced. Thus, in the presence of glucose the rate of reduction of the photooxidized $P700^+$ illuminated for 0.5 sec and more did not reach the control level even with prolonged periods of dark adaptation (Fig. 4b). Possibly, on the short-term of the cells the plastoquinone pool was oxidized incompletely and this increased the rate of the post-illumination reduction of $P700^+$.

Effects of processes on the acceptor side of PSI on kinetics of redox transformations of P700 in *Synechocystis* 6803 cells. NADPH generated on the acceptor side of PSI as a result of the photosynthetic transport of electrons in the thylakoid membrane of *Synechocystis* 6803 is used for fixation of CO_2 in the Calvin cycle and reduction of O_2 to H_2O [11]. The treatment of the cells under aerobic conditions with the inhibitor of CO_2 fixation iodoacetamide (IAC) [18] or transmission of the cells into anaerobic conditions without IAC only slightly decreased the amplitude of the $P700^+$ signal (Fig. 5, b and c). On removal of oxygen from the suspension of IAC-treated cells, the photooxidized $P700^+$ rapidly reduced and remained in the reduced state for 1-2 sec after the illumination was started. As the illumination was continued, the level of $P700^+$ oxidation gradually increased, possibly due to electron transfer onto oxygen generated by PSII in the light (Fig. 5d). Intensive aeration of the cells, which were anaerobically pretreated with IAC, resulted in a very rapid oxidation of $P700^+$, and its oxidized state was maintained until the light was switched off. Under these conditions, the kinetic curve of the EPRI signal virtually lacked phase II (Fig. 5e).

The data indicate that removal of electron acceptors from PSI increases the level of $P700^+$ reduction during uninterrupted illumination of the cells. As in the case of glucose addition, the increase in the level of $P700^+$ reduction in the light was mainly expressed by the decrease in the amplitude of EPRI signal in phase III.

Fig. 5. Effects of IAC and aeration on the redox kinetics of P700 in wild-type *Synechocystis* 6803 cells illuminated with white light for 5 sec with dark intervals of 10 sec: a) control, air; b) control, argon; c) 8 mM IAC, air; d) 8 mM IAC, argon; e) 8 mM IAC, argon, with a subsequent aeration.

DISCUSSION

EPR spectroscopy was used to study the kinetics of light-induced electron transport through PSI in intact cyanobacterial cells. In the present work, we analyzed the electron transport through PSI in *Synechocystis* 6803 exposed to alternating illumination and dark periods using the accumulation of EPR signal from $P700^+$. With this approach, we could use suspensions of actively growing cells with a rather low concentration of chlorophyll (20-40 μg/ml). For a single recording of the EPRI signal the kinetic curve significantly depended on duration of the dark period before the beginning of the cell illumination [19-21]. To study the effects of duration of the light and dark periods, in the present work the cells were preadapted to the corresponding regimen of the light and dark alternation by subjecting them to 10 cycles without recording the signal.

Differences in kinetic curves of the EPRI signal in intact systems (green leaves or cyanobacterial cells) and in isolated chloroplasts were also recorded earlier [19-21]. For intact leaves, a complicated kinetic curve of the $P700^+$ signal was obtained which included all three phases recorded in the present work. In isolated chloroplasts of B class the kinetic curve of the $P700^+$ signal was monophasic. Reasons for these differences were not studied in detail, although it was noted that they seemed to be associated with the oxygen concentration in the cell suspension [19], acidification of the intra-thylakoid space slowing down the electron transfer on the plastoquinone region of ETC [20], and also events on the acceptor side of PSI [21]. In the present work, we attempted to determine the dependence of the EPRI signal kinetic curve on duration of the light and dark periods, presence or absence of respiration substrates, and also on the effects of inhibitors of electron transport on the donor and acceptor sides of PSI.

On the donor side of PSI electrons come onto photooxidized $P700^+$ through the PQ pool and the cytochrome b_6f complex. The PQ pool can be reduced by noncyclic and cyclic electron transport and also due to oxidation of respiration substrates located in the cytoplasm. The production of NADPH in photoheterotrophic cyanobacteria as a result of glucose oxidation in the oxidative pentose phosphate cycle is suppressed in the light with RuDP, a metabolite of the Calvin cycle [4]. In the light, the photosystem II is a chief electron donor for the PQ pool (and then onto $P700^+$), although under certain conditions the contribution of cyclic electron transport can significantly increase [22]. In the presence of DCMU, when electron flow from PSII is inhibited, the electron transfer in the light onto $P700^+$ depends on the activity of cyclic electron transport. Both NADPH- and Fd-dependent cyclic transport of electrons in *Synechocystis* 6803 occur with involvement of the NDH-1 complex [10].

When cells of the photoheterotrophic cyanobacterium *Synechocystis* 6714 from the same taxonomic group as *Synechocystis* 6803 [2] were transmitted into the dark, the fixation of CO_2 was suppressed because some enzymes of the Calvin cycle (i.e., phosphoribulokinase which catalyzes the generation of RuDP) were induced in the light and inactivated in the dark [3]. In the absence of RuDP, G6P dehydrogenase was activated, which was involved in reduction of glucose in the pentose phosphate cycle during which $NADP^+$ was reduced to NADPH [3]. The NADPH concentration rapidly increased in the cells of *Synechocystis* 6714 after their transmission into the dark [23]. Thus, the level of reduction of the PQ pool in the dark due to respiratory electron transport (with involvement of NDH-1 and oxidases) could increase within the first minutes after the switching off the light until the steady state is reached.

The data presented in this work show that an increase in duration of the dark adaptation periods of the *Synechocystis* 6803 cells is associated with appearance in the kinetic curve of the EPRI signal of the $P700^+$ rapid reduction phase in the light (phase II). This can be associated with an enhanced respiratory transport of electrons even within the first seconds of the dark incubation of the cells and, correspondingly, with an increase in the reduction level of the PQ pool, which is a donor of electrons for $P700^+$. Note that in the absence of the PSII activity the monophasic type of the curve was recorded even at long-term periods of the dark adaptation of the cells. Thus, the appearance of phase II in the kinetic curves of the EPRI signal either directly depends on the electron arrival from PSII (together with electrons from respiration substrates) or is due to a high content in the cytoplasm of NADPH produced during noncyclic transport of electrons.

NADPH produced as a result of noncyclic electron transport is oxidized on the acceptor side of PSI during the light-induced fixation of CO_2 and reduction of oxygen to water with involvement of flavoproteins Flv1 and Flv3 [11]. Changes in the kinetic curve of the EPRI signal, such as an increased level of the $P700^+$ reduction during phase III, was recorded only in the case of simultaneous removal of two acceptors of electrons from PSI (on inhibition of the CO_2 fixation with iodoacetamide under anaerobic conditions), which could effectively substitute for each other [11]. Under increased aeration of the cells, phase II virtually disappeared in the kinetic curve of the EPR signal from $P700^+$, and the character of the curve approached monophasicity (Fig. 5e). Based on these findings, it was suggested that the appearance in the kinetic curves of the phase of the rapid reduction of $P700^+$ immediately after the beginning of the illumination could be caused by a lag-phase in the fixation of CO_2 [24] under conditions of insufficient aeration of the cells.

Fixation of CO_2 is induced by light and rapidly ceases in the dark [3]. At least three reactions of the Calvin cycle are light-regulated. Phosphoribulokinase, which

catalyzes RuDP formation, is activated immediately after switching on the light, whereas hydrolysis of fructose-1,6-diphosphate and sedoheptulose-1,7-diphosphate with the corresponding diphosphatases is rapidly inhibited in the dark but reactivated in the light within a few seconds [6]. Thus, NADPH can be oxidized with a low rate in the Calvin cycle because of a decelerated generation of intermediate products of CO_2 fixation. And electrons from PSI are mainly accepted by oxygen, the concentration of which in the quartz cuvette can considerably decrease in the course of dark respiration [19]. The appearance of phase II in the kinetic curve of the EPRI signal is likely to be associated with the delivery of electrons from plastoquinone onto oxidized $P700^+$ when the electron withdrawal onto acceptors of PSI is limited.

In the present work, we have revealed some factors influencing the kinetic curve of the EPR signal from $P700^+$. Obviously, the rate of electron transfer through PSI is determined by the ratio of the electron transport rates on the acceptor and donor sides of PSI. The events on the acceptor and donor sides of PSI, in their turn, can significantly depend on both external factors (e.g., stress conditions) and internal energy status of the cell.

The work was supported by the Russian Foundation for Basic Research (project No. 03-04-49217).

REFERENCES

1. Rippka, R. (1972) *Arch. Microbiol.*, **87**, 93-98.
2. Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M., and Stanier, R. Y. (1979) *J. Gen. Microbiol.*, **111**, 1-61.
3. Pelroy, R. A., and Bassham, J. A. (1972) *Arch. Microbiol.*, **86**, 25-38.
4. Pelroy, R. A., Rippka, R., and Stanier, R. Y. (1972) *Arch. Microbiol.*, **87**, 303-322.
5. Pelroy, R. A., and Bassham, J. A. (1973) *J. Bacteriol.*, **115**, 943-948.
6. Pelroy, R. A., Levine, G. A., and Bassham, J. A. (1976) *J. Bacteriol.*, **86**, 633-643.
7. Jones, L. W., and Myers, J. (1963) *Nature*, **199**, 670-672.
8. Molitor, V., Trnka, M., and Peschek, G. A. (1987) *Curr. Microbiol.*, **14**, 263-268.
9. Ogawa, T. (1992) *Research in Photosynthesis* (Murata, N., ed.) Vol. III, Kluwer Academic Publishers, Dordrecht.
10. Mi, H., Endo, T., Ogawa, S., and Asada, K. (1995) *Plant Cell Physiol.*, **36**, 661-668.
11. Helman, Y., Tchernov, D., Reinhold, L., Shibata, V., Ogawa, T., Schwarz, R., Ohad, I., and Kaplan, A. (2003) *Curr. Biol.*, **13**, 230-235.
12. Cooley, J. W., and Vermaas, W. F. J. (2001) *J. Bacteriol.*, **183**, 4251-4258.
13. Howitt, C. A., Udall, P. K., and Vermaas, W. F. J. (1999) *J. Bacteriol.*, **181**, 3994-4003.
14. Howitt, C. A., and Vermaas, W. F. J. (1998) *Biochemistry*, **37**, 17944-17951.
15. Berry, S., Schneider, D., Vermaas, W. F. J., and Rogner, M. (2002) *Biochemistry*, **41**, 3422-3429.
16. Elanskaya, I. V., Grivennikova, V. G., Groshev, V. V., Kuznetsova, G. V., Semina, M. E., and Timofeev, K. N. (2004) *Biochemistry (Moscow)*, **69**, 137-142.
17. Elanskaya, I. V., Timofeev, K. N., Grivennikova, V. G., Kuznetsova, G. V., Davletshina, L. N., Lukashev, E. P., and Yaminkii, F. V. (2004) *Biochemistry (Moscow)*, **69**, 445-454.
18. Kaplan, A., and Reinhold, L. (1999) *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, **50**, 539-570.
19. Trubitsin, B. V., Mamedov, M. D., Vitukhnovskaya, L. A., Semenov, A. Yu., and Tikhonov, A. N. (2003) *FEBS Lett.*, **544**, 15-20.
20. Tikhonov, A. N., Khomutov, G. B., Ruuge, E. K., and Blumenfeld, L. A. (1981) *Biochim. Biophys. Acta*, **637**, 321-333.
21. Tikhonov, A. N., Ruuge, E. K., Subchinsky, V. K., and Blumenfeld, L. A. (1975) *Fiziol. Rast.*, **22**, 5-14.
22. Jeanjean, R., Bedu, S., Havaux, M., Matthijs, H. C. P., and Joset, F. (1998) *FEMS Microbiol. Lett.*, **167**, 131-137.
23. Pelroy, R. A., Kirk, M. R., and Bassham, J. A. (1976) *J. Bacteriol.*, **86**, 623-632.
24. Buchanan, B. B. (1991) *Arch. Biochem. Biophys.*, **288**, 1-9.