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OXIDATION AND REDUCTION OF PLASTOQUINONE BY PHOTOSYNTHETIC AND RESPIRATORY ELECTRON TRANSPORT IN A CYANOBACTERIUM *SYNECHOCOCCUS* sp.

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The I-D dip, an early transient of the fluorescence induction, was examined as a means to monitor redox changes of plastoquinone in cells of a cyanobacterium, *Synechococcus* sp. That the occurrence of the dip depends upon the reduced state of the plastoquinone pool was indicated by observations that 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone and 3-(3,4-dichlorophenyl)-1,1-dimethylurea did not affect the initial rise to I but abolished the subsequent decline from I to D and that illumination of the cells with light 1, prior to fluorescence measurements, eliminated the transient. The I-D dip was prominent in freshly harvested cells containing abundant endogenous substrates, disappeared slowly as the cells were starved by aeration but reappeared on addition of fructose to the starved cells in the dark. The dip that had been induced by a brief illumination of the starved cells with light 2 was rapidly diminished in the dark and KCN inhibited the dark decay of the transient. The results indicate that plastoquinone is reduced with endogenous as well as exogenous substrates and oxidized by a KCN-sensitive oxidase in the dark, thus providing strong support for the view that plastoquinone of photosynthetic electron transport also functions in respiration. In addition, the occurrence of a cyclic pathway of electrons from Photosystem I to plastoquinone, possibly via ferredoxin or NADP, was suggested. Several lines of evidence indicate that, under a strong light 2, Photosystem I-dependent oxidation of plastoquinone predominates over Photosystem II-dependent reduction of the quinone in the cyanobacterium which contains Photosystem I more abundantly than Photosystem II.

Introduction

Lipophilic quinones carrying isoprenoid side chains play an important role as a carrier of electrons and protons in membrane-bound energy-conservation systems. Higher plants and algae contain two major physiological quinones in different cell compartments specialized in photosynthesis and respiration. Plastoquinone is the functional quinone in electron transport of chloroplasts, whereas mitochondria contain ubiquinone as an intermediate of the respiratory chain.

Cyanobacteria (blue-green algae) which carry out plant-type oxygenic photosynthesis contain plastoquinone, vitamin K-1 and other minor quinones but no ubiquinone [1–3]. The question arises as to which quinone present in cells is involved in respiration of cyanobacteria. Peschek [3] has suggested that vitamin K-1 is the main respiratory quinone in *Anacystis nidulans*, because the quinone restored respiratory activities of pen-

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DBMIB 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; PS, photosystem; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

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tane-extracted particles isolated from the cells. However, the restoration was not specific to vitamin K-1: A significant reactivation of the lost activities was observed on addition of plastoquinone [3].

Eisbrenner and Bothe [4] have shown that DBMIB inhibits the Knallgas reaction and light-induced C_2H_2 reduction of intact filaments and H_2-dependent C_2H_4 formation in heterocysts of *Anabaena cylindrica* to the same extent. Since DBMIB is an inhibitor of plastoquinone oxidation [5] and blocks photosynthetic electron transport in chloroplasts more strongly than respiration in mitochondria [6,7], they proposed that plastoquinone is a common intermediate of photosynthesis and respiration [4]. In other cyanobacteria, however, respiratory activities are significantly more resistant to the poison than light-induced electron transport [8,9].

The role of plastoquinone in a cyanobacterium, *Synechococcus* sp., was more explicitly demonstrated by Hirano et al. [8] who monitored the redox state of the quinone in situ by measuring reduction kinetics of flash-oxidized cytochrome having the α-band maximum at 553 nm. The observations that the cytochrome reduction was slowed down by the starvation of cells but accelerated by the addition of substrates, such as fructose and glucose or an inhibitor of respiration, KCN, strongly suggest that plastoquinone is reduced with respiratory substrates and oxidized by a KCN-sensitive oxidase.

Another kinetic approach in monitoring redox changes of plastoquinone in *Synechococcus* cells is presented in this work. An early transient of the fluorescence induction, the I-D dip [10–15], was employed to determine the redox state of the quinone in situ. An advantage of the fluorescence method over the repetitive flash spectroscopy used for the determination of cytochrome kinetics is that the redox state of the quinone can be determined much more quickly, thus enabling us to measure kinetics of plastoquinone changes in the second to subsecond time range. Oxidation and reduction of plastoquinone during illumination of PS I and PS II, as well as in the dark, were analyzed. The results strongly support the view that plastoquinone is a common link between photosynthetic and respiratory electron transport in the cyanobacterium.

Materials and Methods

The thermophilic cyanobacterium, *Synechococcus* sp., was grown photoautotrophically at 55°C for 24 h as described previously [8,16]. Cells were harvested by centrifugation and suspended in a fresh growth medium containing 25 mM Hepes-NaOH (pH 7.5) at about 10 μg chlorophyll/ml for fluorescence measurements. Fluorescence was excited with broad-band red light (570–620 nm) which passed through a water filter of 4 cm thickness, a Toshiba V-O 57 cut-off filter and a Vacuum Optics DM filter and was measured by a photomultiplier (Hamamatsu TV. R236) through a V-R 67 cut-off filter and an interference filter with maximum transmission at 688 nm. The intensity of the excitation light was $1.9 \cdot 10^5$ erg $\cdot$ cm$^{-2} \cdot$ s^{-1}. Signals were stored in a Rikendenshi TCCD 2000 transient converter and displayed on a Hitachi 056-1001 recorder. Where indicated, the cells were illuminated, prior to fluorescence measurements, with 620 nm light of $1.2 \cdot 10^4$ erg $\cdot$ cm$^{-2} \cdot$ s^{-1} which is mainly absorbed by phycobilins (light 2), or 430 nm light of $5 \cdot 10^3$ erg $\cdot$ cm$^{-2} \cdot$ s^{-1} which preferentially excites chlorophyll *a* (light 1). Appropriate combinations of cut-off and interference filters were used to obtain the two monochromatic light beams. Light sources were Nikon microscope illuminators with a 100 W halogen lamp. All experiments were carried out at 55°C.

Results

The I-D dip

Time courses of the fluorescence induction in freshly harvested cells of *Synechococcus* sp. are shown in Fig. 1. Illumination with strong red light induced a rapid rise to a high level I, then a quick drop to a low level D, followed by slower changes (trace a). The early transient has been reported in various algae [10–13] and intact chloroplasts isolated from spinach [14] and a green alga [15] and has been designated the I-D dip by Munday and Govindjee [11]. The dip was prominent in algal cells which had been incubated for long periods

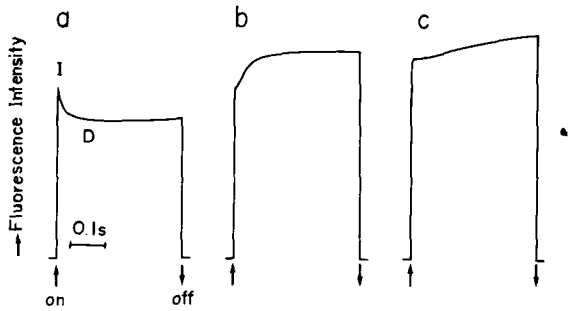

Fig. 1. Fluorescence induction in freshly harvested cells. After 24 h of growth, cells were collected by centrifugation and suspended in a growth medium containing 25 mM Hepes-NaOH (pH 7.5). For the fluorescence measurement, cells were kept at 55°C by circulating heated water through a water jacket of cuvettes and were illuminated with broad-band red light (570–620 nm) of $1.9 \cdot 10^5$ erg·cm^{-2}·s^{-1}. a, no addition; b, 10 μM DBMIB and 5 mM ascorbate; c, 10 μM DCMU.

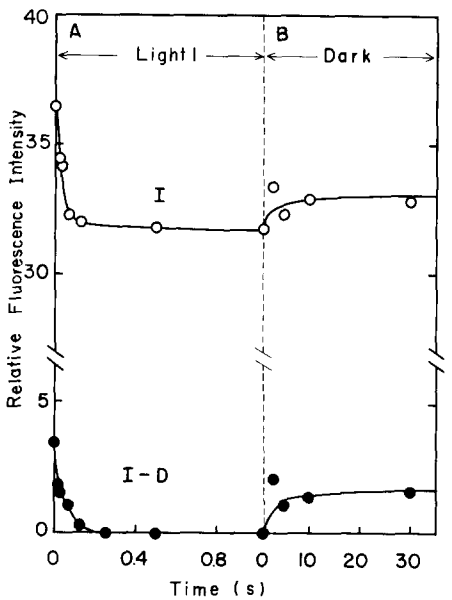

Fig. 2. Disappearance of the I-D dip in light 1. (A) Freshly harvested cells were illuminated with 430 nm light ($5 \cdot 10^3$ erg·cm^{-2}·s^{-1}) for the indicated periods prior to the fluorescence measurement. The duration of illumination was varied with a camera shutter. (B) After illumination for 1 s, cells were kept in the dark and the fluorescence transient was determined at the indicated times. Different time scales were used in A and B. Other experimental conditions were as described in Fig. 1. Open circles, heights of I. Closed circles, magnitudes of the I-D drop.

under dark anaerobic conditions [12,13] or in intact chloroplasts which had been treated with dithionite and illuminated briefly [14,15]. These treatments accumulate the reduced form of secondary electron acceptors of PS II. The fluorescence yield is determined by the redox state of Q, the electron acceptor of PS II [17], which in turn is controlled by a balance between its reduction by PS II and its oxidation by the plastoquinone pool. Thus, the initial rise to I indicates a rapid reduction of Q due to the absence of electron transfer to the plastoquinone pool present in the reduced state. The subsequent drop from I to D arises as plastoquinone is oxidized by electron transport to PS I.

In contrast to algae, the dip was most marked when the cyanobacterium had been kept for a long period in the light. Thus, the transient was pronounced in freshly harvested cells but gradually disappeared as the cells were left in the dark. Nevertheless, the mechanism underlying the fluorescence transient is essentially the same as that for the I-D dip in the dark anaerobic cells of algae. DBMIB which inhibits electron transport from plastoquinone to PS I [5] did not affect the initial rise to I but replaced the subsequent drop from I to D by an increase to the steady-state level (trace b). The poison was added with an excess of ascorbate to eliminate the quenching effect of its oxidized form. The I level was elevated and the I-D drop was abolished in the presence of DCMU, an inhibitor of electron transport from Q to plastoquinone (trace c). The I level was not affected by DCMU in cells which exhibited a more prominent dip. The results indicate that a significant part of the plastoquinone pool is present in the reduced state and that the I-D drop is due to the quinone oxidation by PS I. A slow fluorescence increase in the presence of DCMU has been observed previously in *A. nidulans* and related to a change in the excitation energy distribution [18].

Effect of light 1

Fig. 2 presents more evidence for the dependence of the I-D dip on the reduced state of the plastoquinone pool. The cells, freshly harvested, were irradiated with 430 nm light for different lengths of time and the fluorescence transient was

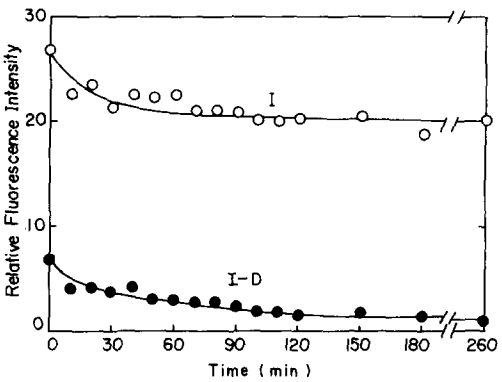

Fig. 3. Disappearance of the I-D dip during dark starvation cells. Freshly harvested cells were aerated in the dark at 55°C. Other experimental conditions were as in Fig. 1. Open circles, heights of I. Closed circles, magnitudes of the I-D drop.

determined within 1 s after the light was turned off. Plastoquinone is expected to be oxidized by the illumination with the 430 nm light, which mainly excites chlorophyll *a*. It can be seen that the height of I decreased rapidly to reach a constant level within 0.2 s of the illumination. The magnitude of the I-D drop was reduced in parallel. The I level and the I-D drop were partially restored in the dark. Thus, the height of I and magnitude of the fluorescence decrease from I to D are both correlated with the reduced state of the plastoquinone pool.

Dark starvation of cells

Fig. 3 shows that the I-D dip also disappeared when the cells had been aerated in darkness. A slow decrease in the I level in the dark has been observed previously by Mohanty and Govindjee [18]. Hirano et al. [8] have found that the reduction kinetics of flash-oxidized cytochrome *c*-553 were gradually slowed down in parallel with lowering of endogenous respiration during the dark aeration of *Synechococcus* cells. They suggested that plastoquinone is reduced with endogenous substrates and the oxidized form of the quinone accumulates only after an extensive starvation of the cells. The extremely slow disappearance of the fluorescence transient is compatible with this explanation. A few hours of dark aeration were needed to eliminate the I-D dip in the organism

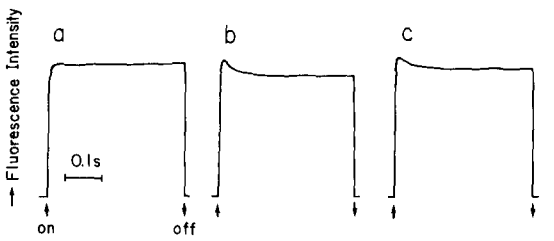

Fig. 4. Fluorescence induction in starved cells. Cells were starved by aeration in the dark at 55°C. In this experment, 1 h of dark aeration was sufficient to diminish the I-D dip as shown in a. The starved cells were incubated for 5 s in darkness with 20 mM fructose (b) or 1 mM KCN (c). Other conditions, see Fig. 1.

employed here. The dark restoration of the dip observed after illumination with light 1 also suggests the reduction of plastoquinone with a reductant present in the cells.

Effects of fructose and KCN

The reduction of plastoquinone with a reductant was more directly demonstrated by adding a substrate of respiration to the starved cells. Fig. 4 shows the kinetics of the fluorescence induction in starved cells. The fluorescence yield remained at a low level without showing any significant transient feature, indicating that Q was mostly kept in the oxidized state during illumination (trace a). Fructose serves as a substrate for respiration of the organism [8]. The incubation of starved cells with the sugar for 5 s in darkness induced an appreciable extent of the I-D (trace b).

The fluorescence transient also appeared after the incubation of starved cells with KCN which inhibits respiration of the organism [8]. Our interpretation is that plastoquinone is slowly reduced with a residual level of endogenous substrates on the one hand and is oxidized by the respiratory chain with O_2 as electron acceptor on the other in the starved cells. Thus, the inhibition of the terminal oxidase of respiration by KCN results in a reduction of plastoquinone. The KCN effect cannot be ascribed to inactivation of plastocyanin, because the copper protein is absent from *Synechococcus* cells [19].

The time courses of changes in the I level and the magnitude of the I-D drop in the presence of

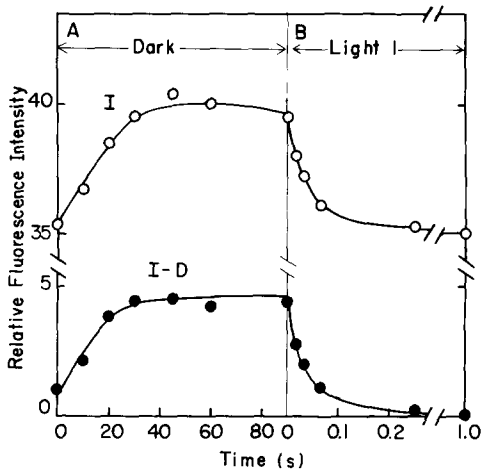

Fig. 5. The time course of the development of the I-D dip in the presence of fructose and KCN and the subsequent disappearance of the transient in light 1. Cells starved for 3 h were incubated with 20 mM fructose and 1 mM KCN in the dark (A), and after steady-state levels were attained light 1 was turned on as in Fig. 2B. Open circles, heights of I. Closed circles, magnitudes of the I-D drop. Other conditions, see Fig. 1.

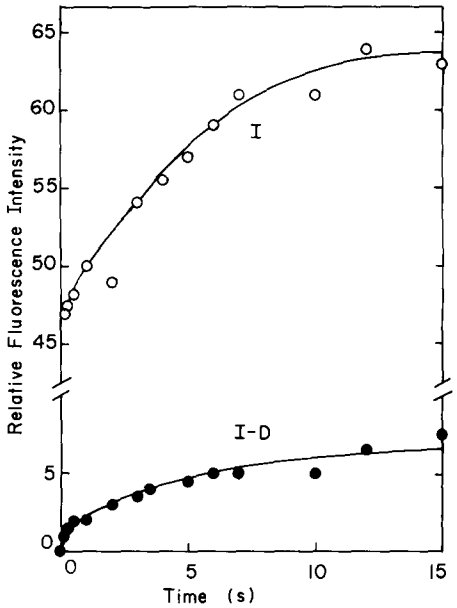

Fig. 6. The development of the I-D dip in light 2. Cells starved for 3 h were illuminated with 620 nm light ($1.2 \cdot 10^4$ erg·cm^{-2}·s^{-1}) for the indicated periods prior to the fluorescence measurement. Other conditions, see Fig. 1. Open circles, heights of I. Closed circles, magnitudes of the I-D drop.

fructose and KCN are illustrated in Fig. 5. The I-D dip was fully developed after 30 s of incubation. The subsequent illumination with light 1 eliminated the dip quickly and completely. We conclude that the endogenous substrate and PS I affect the same pool of plastoquinone. Thus, the substrate donates electrons to PS I via plastoquinone.

Dark oxidation of plastoquinone

The above observation that the dip could be induced by the incubation of starved cells with KCN suggests plastohydroquinone oxidation by the respiratory oxidase. However, the data may also be explained in terms of competition for a common reductant between plastoquinone and the oxidase: The reductant that may be produced slowly from endogenous substrates is mostly consumed by respiration but is able to reduce plastoquinone when respiration is blocked with KCN. The following experiments were undertaken to examine whether or not plastoquinone is indeed oxidized in the dark in a KCN-sensitive way. Starved cells were employed to minimize interference by endogenous substrates and plastoquinone was reduced by brief illumination with light 2.

Effects of light 2 on the development of the I-D dip were first studied (Fig. 6). The cells were irradiated with 620 nm light for the indicated periods, then the second illumination was given to measure the fluorescence transient with a 1 s dark interval in between. The illumination with light 2 induced a marked increase in the I level. Note that the increase in the height of I far exceeded the magnitude of the I-D drop. This indicates that light 2 caused an additional fluorescence increase which cannot be ascribed to redox changes of Q but may be related to the slow fluorescence increase observed in the present of DCMU (see Fig. 1). The extent of plastoquinone reduction was therefore monitored by the magnitude of the I-D drop. It can be seen that quinone reduction proceeded slowly in light 2. More than 15 s of illumination were required to attain the maximum extent of the transient.

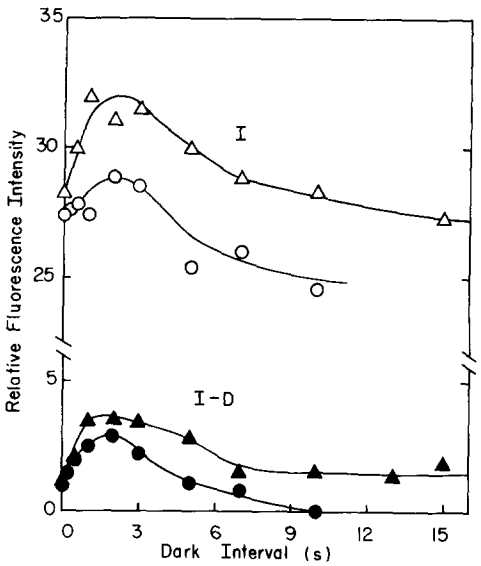

Fig. 7. The postillumination changes of the I-D dip. Cells starved for 3 h were illuminated for 5 s with light 2 as in Fig. 6 and the I-D dip was determined after varied periods of the dark interval. Open circles and triangles, heights of I determined in the absence and presence of 1 mM KCN, respectively. Closed circles and triangles, magnitudes of the I-D drop determined in the absence and presence of KCN, respectively.

The dark oxidation of plastoquinone photoreduced by the illumination with light 2 for 5 s was determined by varying the duration of the dark interval between the first actinic and the second measuring illumination (Fig. 7). Unexpectedly, the I-D dip was still insignificant immediately after cessation of the first illumination but grew rapidly in the dark. Thus, light 2 is quite ineffective in photoreducing plastoquinone. However, the illumination seems to accumulate a reductant for the quinone. The dark growth of the dip was enhanced when KCN had been added just before the first illumination. The postillumination growth ceased after 2 s, presumably reflecting a small quantity of the reductant produced during the first illumination. Then, the I-D dip decreased rapidly. This shows that dark oxidation of plastoquinone. The half-time of the dark decay was about 3 s and the dip disappeared completely 10 s after cessation of the first illumination. The dark decay of the I-D dip was sensitive to KCN. Although the inhibition was not complete, a significant magnitude of the fluorescence transient occurred persistently after prolonged dark intervals in the presence of the poison.

Discussion

Role of plastoquinone

The present work demonstrates that the I-D dip is a good measure of the redox state of plastoquinone in situ. The I-D dip occurs at the earliest stage of the fluorescence induction and is completed within a small fraction of 1 s with the intense excitation light used. Thus, slower fluorescence transients due to changes in the energy state of the thylakoid membranes or the excitation transfer between the two photosystems (see Ref. 20 for a review) should not significantly interfere with monitoring the redox state of the quinone through the I-D dip.

The I-D dip senses the plastoquinone pool, which accepts electrons from Q and then gives them to PS I. The findings that the dip induced by a brief illumination with light 2 disappeared quickly in the dark and that the dark decay of the transient was suppressed by KCN strongly suggest, therefore, that the plastoquinone pool functioning in photosynthesis is oxidized through the terminal oxidase of respiration. The dark oxidation of plastoquinone proceeded much more slowly than did PS I-dependent oxidation of the quinone. This is compatible with the previous observation that the rate of dark respiration is only a few percent of that of photosynthetic electron transport [8].

Various organic and inorganic compounds donate electrons to PS I in cyanobacteria [3,4,21,22]. The participation of plastoquinone in this span of electron transport was clearly demonstrated by the appearance of the dip on addition of fructose to starved cells and the subsequent disappearance of the transient under illumination with light 1.

The I-D dip was prominent in *Synechococcus* cells that had been illuminated for long periods. This is explained in terms of plastoquinone reduction with endogenous substrates photosynthetically produced. On the other hand, the dip was promoted by prolonged incubation of cells under

dark anaerobic conditions in eukaryotic algae [12,13]. This also implies that plastoquinone is slowly reduced with an endogenous reductant and oxidized with O_2 in the thyalkoid membranes, which are separated from mitochondria by the outer limiting membrane (envelope). These dark redox changes of electron carriers between the two photosystems would deserve more attention from the viewpoint of the symbiotic hypothesis, which assumes an ancestor of cyanobacteria as the origin of the contemporary chloroplasts [23].

The I-D dip provides only qualitative information concerning the redox state of the plastoquinone pool. More quantitative analyses of the pool are in progress. However, as the fluorescence transient senses the reduced state of the pool from the PS II side, the results are complementary to those obtained from kinetic studies of cytochrome *c*-553 reduction which view the pool from the PS I side [8]. The agreement between the previous and present study tends to suggest that the plastoquinone pool functioning in photosynthesis is entirely shared by respiration. A previous work has shown that plastoquinone serves as a mobile electron carrier through the fluid hydrophobic matrix of the thylakoid membranes [24].

Effects of light 2

Light 2 was found to exert complex effects on the redox state of plastoquinone. Illumination of the starved cells with light 2 was quite ineffective in accumulating plastohydroquinone. This contrasts with the rapid and complete oxidation of the quinone in light 1. On the other hand, the fluorescence induction determined in the presence of DBMIB indicates that plastoquinone could be reduced rapidly by PS II (see Fig. 1). The interpretation is that plastoquinone is reduced by PS II but PS I functions to keep the quinone in the oxidized state even in light 2. The occurrence of the I-D dip is an indication of the operation of PS I in light 2, because the light used for the fluorescence excitation is mainly absorbed by phycobilins. Q was kept mostly in the oxidized state in the starved cells under the excitation illumination (Fig. 4a). Thus, strong light 2 acts as if it were light 1 in respect to the steady redox state of Q and plastoquinone in the cyanobacterium.

It is known that in cyanobacteria the reaction center of PS I is more abundant than the reaction center of PS II [25,26]. The organism employed here shows a ratio of about three PS I reaction centers to one PS II reaction center [19]. The PS I-dependent oxidation of plastoquinone may surpass its reduction by PS II in strong light 2 when a significant portion of the excitation energy is directed to the PS I reaction center through the spillover from the pigments of PS II and the rate of electron transfer from plastoquinone to cytochrome *f* is sufficiently high. The plastoquinone oxidation that is the rate-limiting reaction of photosynthetic electron transport seems to proceed much more rapidly in the cyanobacterium than in higher plants and algae. Hirano et al. [24] have shown that the half-reduction time of cytochrome *c*-553 with electrons from plastoquinone is less than 2 ms at physiological temperatures for the thermophilic cyanobacterium [24].

Rapid reduction of plastoquinone occurred immediately after the illumination with light 2. This suggests the production of a reductant during the illumination. Postillumination reduction was seen to an enhanced extent in the presence of KCN. Because KCN inhibits the CO_2 reduction, the occurrence of postillumination reduction in the poisoned cells indicates that the reductant produced by light 2 is not a product of photosynthetic CO_2 fixation. Instead, the results suggest the operation of a cyclic pathway of electrons: plastoquinone reduction with products of photosynthetic electron transport, such as reduced ferredoxin or NADPH.

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