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Electrons generated by photosystem II are utilized by an oxidase in the absence of photosystem I in the cyanobacterium *Synechocystis* sp. PCC 6803

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Abstract

The reduction and reoxidation kinetics of the first quinone-type electron acceptor in photosystem II, Q_A^-, were measured by fluorescence in a light-tolerant, photosystem I-less strain of the cyanobacterium *Synechocystis* sp. PCC 6803. In this strain, which shows excellent amplitudes of variable fluorescence, the rate of Q_A^- oxidation after photoreduction of the plastoquinone pool was about half of that in the presence of photosystem I. However, upon addition of 5 mM KCN, Q_A^- decay was very slow, and the rate was comparable to that seen in the presence of diuron, which blocks electron transport between Q_A and Q_B. The KCN-imposed block of Q_A^- oxidation was removed efficiently by addition of exogenous quinones that can oxidize the plastoquinone pool. These results indicate that, in the absence of photosystem I, photosystem II-generated electrons are used very effectively by an oxidase located in the thylakoid; this oxidase may be a component of the respiratory chain.

Key words: Thylakoid membrane; Photosynthesis; Electron transport; Plastoquinone; Respiration; KCN-sensitive oxidase

1. Introduction

In cyanobacteria important parts of both the photosynthetic apparatus and the respiratory chain are located in the thylakoid membrane [1–3]. This has led to the realization that in these organisms an interplay exists between photosynthetic and respiratory electron transport, and that the two electron transport chains may share common components, such as the plastoquinone pool and the cytochrome b_6f complex [1,3]. In spite of the attractiveness of this idea, it has been difficult to quantify the rates and capacities with which electrons from one transport chain can be accommodated in the other.

During the last year, we have developed a *Synechocystis* 6803 strain that lacks photosystem I (PS I) and that can be grown in continuous, dim light ($5~\mu E \cdot m^{-2} \cdot s^{-1}$)

[4]. This strain was obtained by genetic deletion of the PS I core complex upon transformation with a plasmid in which part of the *psa*AB operon (coding for the PS I core complex) was deleted and replaced with an antibiotic-resistance cassette. Resulting transformants were segregated under dim-light conditions to genetic homogeneity with regards to deletion of part of the *psa*AB operon. The PS I-less (*psa*AB-deletion) strain does not survive under light conditions routinely used for wild-type cultures (continuous light at $40~\mu E \cdot m^{-2} \cdot s^{-1}$), and is therefore light-sensitive. However, this strain is the only PS I-less cyanobacterial strain known to date that can grow in continuous $5~\mu E \cdot m^{-2} \cdot s^{-1}$ light (see [4]). Deletion of *apc*E, the gene for the phycobilisome anchor protein, leads to a loss of functional phycobilisomes, and to a virtual elimination of the light sensitivity; the PS I-less/*apc*E⁻ strain can be propagated under light conditions used for wild-type [4]. This implies (i) that reducing equivalents produced by photosystem II (PS II) can be dealt with appropriately in the absence of PS I, and (ii) that the reason for light sensitivity of PS IC-less mutants is related to the amount of reducing equivalents generated by PS II.

An important question is what the sinks of PS II-generated electrons are in the absence of PS I: direct reduction of NADP or NAD by the plastoquinone pool is thermodynamically unfavorable. Instead, oxidases

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(cytochrome oxidases and/or quinol oxidases) would be likely sinks for reducing equivalents produced by PS II. We set out to measure the rate of electrons leaving the PS II complex by monitoring variable fluorescence upon actinic illumination and upon turning off the light. Q_A, the first electron-accepting plastoquinone in photosystem II, in its oxidized form is a quencher of fluorescence, while Q_A^- is not [5].

2. Materials and methods

Generation of the *Synechocystis* 6803 strains lacking PS I (PS I-less) and lacking PS I as well as the phycobilisome anchor protein (PS I-less/apcE$^-$) has been described in [4]. Wild-type and PS I-less/apcE$^-$ *Synechocystis* 6803 liquid cultures were grown in BG11 medium [6] supplemented with 5 mM glucose, at 30°C at 40 μE · m^{-2} · s^{-1} light intensity. Aeration was provided by gentle shaking. The PS I-less strain was grown under the same conditions, but with the flask wrapped in two layers of bench paper. The light intensity inside the flask containing the PS I-less culture was approximately 5 μE · m^{-2} · s^{-1}. Strains were propagated on agar plates in the presence of 5 mM glucose at 30°C at light intensities similar to those used for liquid cultures.

Cells of wild-type and mutants were harvested during the logarithmic growth phase (OD$_{730}$ 0.3–0.6). Thylakoids were prepared from the three strains as described in [7]. Fluorescence emission spectra of thylakoids were measured at 77 K in thylakoid buffer (25 mM HEPES/NaOH pH 7.5, 10 mM MgCl$_2$, 30 mM CaCl$_2$, 10% (v/v) glycerol, and 0.5% (v/v) dimethyl sulfoxide) using a Perkin Elmer LS 50 spectrometer; the excitation wavelength was 435 nm. The concentration of thylakoids corresponded to 1 μg/ml chlorophyll for the PS I-less strains, and to 5 μg/ml for wild-type and the PS II-less psbDIC/DII$^-$ mutant [8].

Induction and decay of chlorophyll fluorescence in intact cells and isolated thylakoids were measured as in [9]. Measurements were performed on a commercial fluorometer (Walz, Effeltrich, Germany) using modulated light pulses for detection of the fluorescence yield. By this method, the variable fluorescence yield can be measured independent from actinic illumination. For data acquisition and analysis, manufacturer-supplied software (DA100) was utilized. The chlorophyll concentration used was 2 μg/ml for PS I-less strains, and 10 μg/ml for wildtype. These chlorophyll concentrations correspond to a PS II concentration that is very similar in suspensions of the three strains [4]. The dark adaptation time before each induction curve was 2 min. During this dark-adaptation time, also the modulated light pulses for measuring fluorescence were turned off.

3. Results

3.1. Fluorescence emission spectra

To verify the absence of PS I in the PS I-less (*psaAB* deletion) strain [4], the low-temperature fluorescence emission spectra of thylakoids from wild-type, PS I-less, and PS II-less strains were recorded upon 435 nm excitation (Fig. 1). In wild-type, the 725 nm emission peak, arising from PS I, is most prevalent, and smaller peaks at 685 and 695 nm are attributed to PS II. In the *psbDIC/* DII$^-$ mutant, lacking the genes for D2 and CP43 and not accumulating the other major PS II proteins in its thylakoids [8], only the 725 nm peak is present, and the 685 and 695 nm peaks have disappeared, in agreement with the assignment of the 685 and 695 nm peaks to PS II. In the PS I-less strain, the 725 nm peak is absent, and the 685 and 695 nm maxima remain. This indeed is indicative
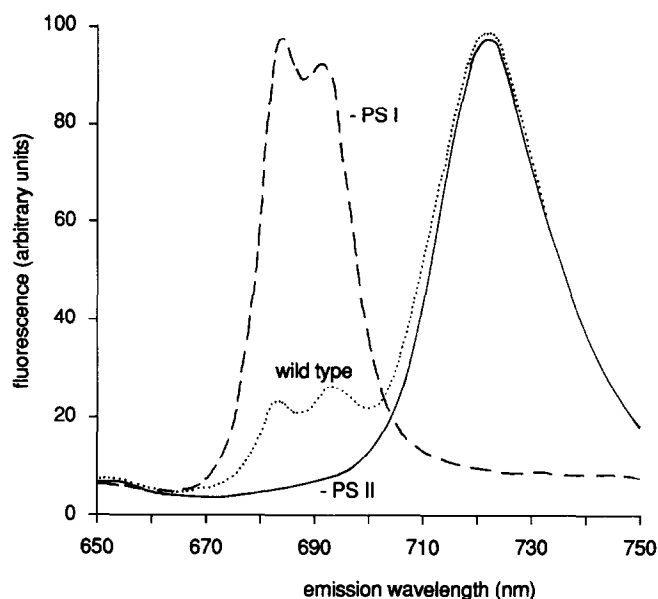

Fig. 1. 77 K fluorescence emission spectrum of thylakoids from wild-type (⋯), the PS II-less *psb*DIC/DII-deletion mutant (—), and the PS I-less (*psa*AB-deletion) mutant (- - - -). The excitation wavelength was 435 nm. Bandwidths of the excitation and emission monochromators were 5 and 1 nm, respectively. The chlorophyll concentration was 5 μg/ml for wild-type and the PS II-less strain, and 1 μg/ml for the PS I-less strain.

of an absence of PS I, and the presence of normal PS II in this strain.

3.2. Variable fluorescence

To measure the kinetics of Q_A reduction and reoxidation in whole cells, fluorescence induction on relatively short timescales (1 s) was measured. During this time, changes in fluorescence yield reflect mostly changes in the redox state of Q_A, and are not yet affected by pH-promoted quenching of chlorophyll fluorescence (see [10] for a review). Fluorescence induction curves of whole cells from wild-type, the PS I-less strain, and the PS I-less/apcE$^-$ strain are presented in Fig. 2. The induction curve of wild-type is rather usual for a cyanobacterium, and has a small F_v/F_o ratio (F_v representing the amount of variable fluorescence, and F_o the amount of chlorophyll fluorescence after dark adaptation). However, in the PS I-less strain the F_v/F_o ratio has increased about 8-fold, and in this strain the F_v/F_o ratio (3.5–4) and the shape of the fluorescence induction curve is rather similar to that seen in isolated, stacked thylakoids from plants [10]. In cells of the PS I-less/apcE$^-$ strain the F_v/F_o ratio is somewhat less favorable than in the PS I-less strain. This may be caused in part by a small functional antenna and by the presence of some phycobilisome components that are not connected functionally to PS II in the thylakoids [4,11].

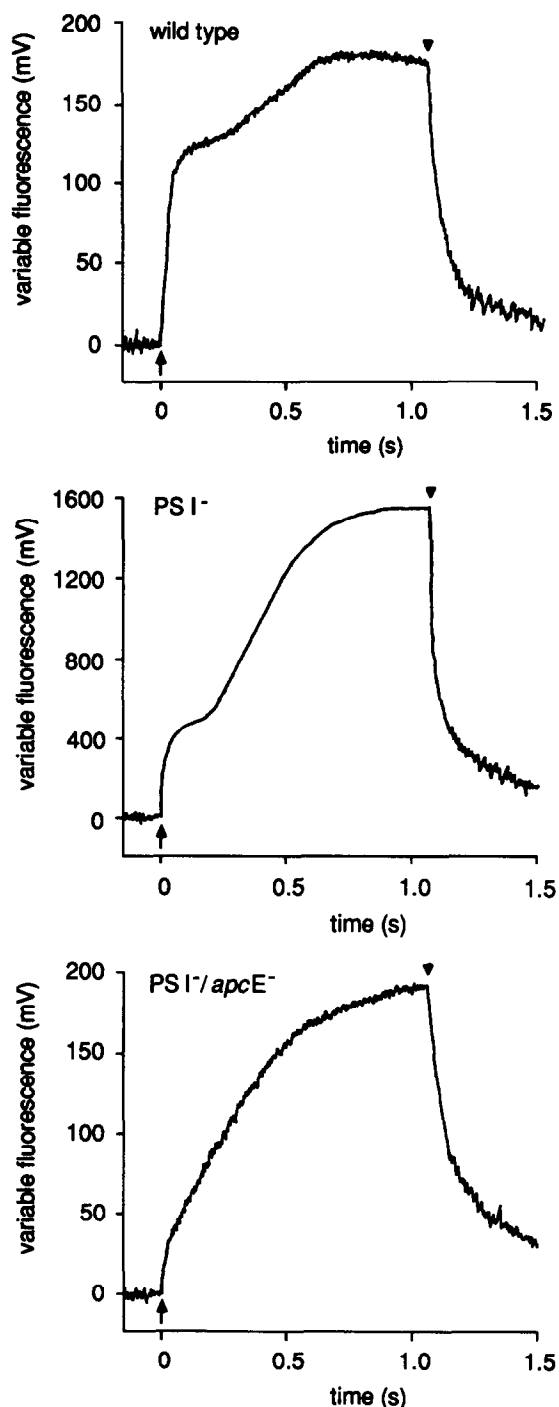Upon turning off the actinic light, Q_A^- is reoxidized

Fig. 2. Fluorescence induction kinetics in whole cells of wild-type (top), the PS I-less mutant (middle), and the PS I-less/apcE⁻ mutant (bottom). The chlorophyll concentration was 10 μg/ml for wild-type, and 2 μg/ml for the two mutants lacking PS I. The fluorescence signal after dark adaptation in the absence of actinic illumination (F_o) was approximately 400 mV in all strains. Actinic light was turned on at time 0 (indicated by the arrow). The decay of the variable fluorescence yield was followed upon turning off actinic illumination (marked by an arrowhead).

very quickly in both the wild-type and PS I-less systems (Fig. 2). This is not surprising for wild-type, but the decay kinetics merit further investigation in the PS I-less

strains. As the F_v/F_o ratio of the PS I-less strain retaining functional phycobilisomes is higher than that in the PS I-less/apcE⁻ strain, we have utilized the PS I-less strain with intact apcE for the following experiments. In Fig. 3, the decay of the variable fluorescence in the PS I-less strain is presented. In the absence of any additions, the half-time of decay of variable fluorescence is approximately 30 ms (Table 1). This is only two-fold slower than the rate found in wild-type (Table 1). The Q_A^- oxidation rate does not necessarily correspond quantitatively with the rate of decay of variable fluorescence, because of a non-linearity in many systems between variable fluorescence yield and relative concentration of Q_A^-. Also in cyanobacteria such a non-linearity probably exists [12], but is relatively small and the degree of non-linearity is of little consequence to our arguments.

In the presence of DCMU, an inhibitor of electron transport between Q_A and the PQ pool, the Q_A^- oxidation rate is slowed down significantly (half-time of variable fluorescence is about 600 ms; Table 1) in both wild-type and the PS I-less strain (Fig. 3). Q_A^- oxidation under these conditions proceeds through back reaction with the water-splitting system [13,14]. Surprisingly, when 5 mM KCN is presented to the cells, in the PS I-less mutant (Fig. 3), but not in the wild-type (Table 1) the Q_A^- oxidation rate is very similar to the rate in the presence of DCMU. This indicates that in the absence of PS I electron transport out of the PQ pool can be inhibited completely by KCN. To verify that the KCN effect is beyond the PQ pool, and is not caused by effects at the level of electron transport between Q_A and Q_B [15], 0.5 mM 2,6-dimethyl-p-benzoquinone was added, which is an effective electron acceptor from the PQ pool in Synechocystis. In the presence of KCN and the quinone, the decay of variable fluorescence in the PS I-less strain was rapid again (Fig. 3 and Table 1), indicating that the KCN effect indeed is unrelated to PS II itself.

These results indicate that in the absence of PS I only KCN-sensitive pathways exist to accept electrons from

Table 1

Half-time of decay of variable fluorescence (Q_A^- oxidation) upon turning off actinic illumination

	Half-time of fluorescence decay (Q_A^- oxidation) in ms	
	Wild type	PS I-less
no additions	14	29
+ KCN	17	690
+ DMCU	600	640
+ KCN + DMBQ	14	34

Cells from wild-type and the PS I-less mutant were illuminated with actinic light for 1 s. During the subsequent 'dark' period, the decay of the variable fluorescence yield was measured using weak (non-actinic) light pulses. The cell concentration corresponded to 2 μg/ml chlorophyll for the PS I-less mutant, and to 10 μg/ml for the wild-type. KCN (5 mM), DCMU (25 μM), and DMBQ (0.5 mM) were added as indicated.
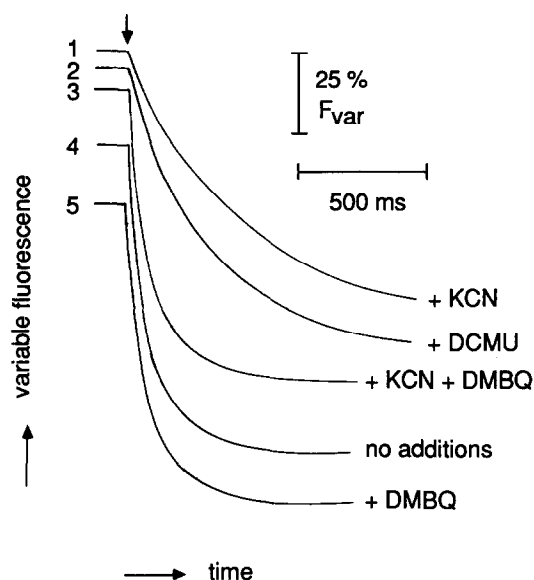
Fig. 3. Decay kinetics of the variable fluorescence yield in intact cells of the PS I-less mutant upon termination of actinic illumination. The chlorophyll concentration was 2 μg/ml, and cells were illuminated for 1 s (as in Fig. 2), after which actinic illumination was turned off, and the variable fluorescence yield was followed. Additions made were as follows: (trace 1) 5 mM KCN; (trace 2) 25 μM DCMU; (trace 3) 5 mM KCN + 0.5 mM DMBQ; (trace 4) no additions; (trace 5) 0.5 mM DMBQ.

PS II. To investigate whether indications could be obtained regarding the number of different KCN-sensitive pathways, the rate of decay of variable fluorescence was measured as a function of KCN concentration. Progressive inhibition of Q_A^- oxidation occurs in the range of 20 μM to 1 mM KCN (data not shown). The KCN inhibition curve of Q_A^- oxidation does not present any evidence for multiphasic behavior, and thus we suggest that PS II electrons may be funneled into a single, KCN-sensitive, electron sink.

To further investigate the effects of KCN on Q_A in the PS I-less strain, fluorescence induction kinetics were measured in this strain in the presence of various compounds (Fig. 4). In the presence of KCN, the F_o level is increased significantly in darkness, and fluorescence induction follows essentially DCMU-type kinetics. The effect of KCN on the redox state of Q_A and of the PQ pool can be countered by addition of an oxidizing quinone; addition of 0.25 mM 2,6-dimethyl-p-benzoquinone (DMBQ) leads to a restoration of essentially normal fluorescence induction features in the PS I-less strain. It appears that, in the presence of an inhibitor of the oxidase, the PQ pool as well as Q_B become totally reduced, probably by action of an NAD(P)H dehydrogenase in the thylakoid [16,17] or of enzyme complexes with similar function; complete reduction of Q_B and the PQ pool results in partial reduction of Q_A as well, resulting in an increased F_o level. Also, note that the maximal fluorescence level in the presence of KCN is a little higher than

that in the presence of DCMU. This difference presumably represents a difference of the redox state of the PQ pool (oxidized in the presence of DCMU; reduced in the presence of KCN).

The KCN effect on the redox state of the PQ pool in darkness should not be typical only for the PS I-less strain, but also would be expected to occur in wild-type. To test this, the effects of KCN and DCMU on fluorescence induction kinetics in wild-type were measured. As shown in Fig. 5, indeed also in wild-type the addition of 5 mM KCN leads to an increase of the F_o level in darkness. After illumination, in the presence of KCN the fluorescence level is found to drop to a value lower than the initial F_o level. The F_o level rises in darkness to the initial level of F_o in 20–40 s (not shown). This indicates that the PQ pool and Q_B are reduced in darkness, and that Q_A is partially reduced under these conditions, just as observed in the PS I-less strain. As soon as the light is turned on, the remainder of Q_A is reduced, but at the same time PS I starts pulling electrons out of the PQ pool, and overall the redox state of Q_A becomes more oxidized than at the start of the illumination. Note that the number of PS I reaction centers exceeds that of PS II in cyanobacterial thylakoids [18]. As soon as the illumination is stopped, Q_A^- is fully oxidized by the PQ pool; this brings the F_o level down to a value comparable to that seen in wild-type after dark adaptation in the absence of KCN.

4. Discussion

A major insight gleaned from these experiments is the efficient contact between PS II and a KCN-sensitive ox-
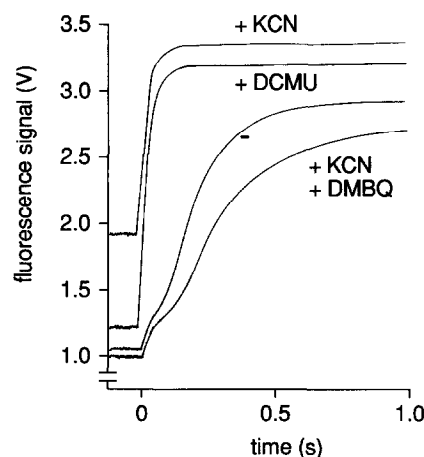

Fig. 4. Fluorescence induction in intact cells of the PS I-less mutant. Additions made one minute before turning on the actinic light have been indicated in the figure (KCN, 5 mM; DCMU, 25 μM; DMBQ, 0.5 mM; –, no additions). The amplitude of the fluorescence signal indicated involves both F_o and F_v. Note the high apparent F_o value in the presence of KCN without DMBQ.
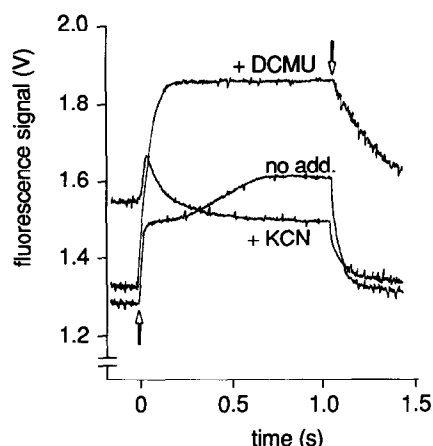
Fig. 5. Fluorescence induction in intact cells of wild-type. Additions made one minute before turning on the actinic light have been indicated in the figure (KCN, 5 mM; DCMU, 25 μM). The moment the actinic light was turned (time 0) has been indicated by an upward arrow. The instant the light was turned off has been marked by a downward arrow.

idase in *Synechocystis*. The rate of PS II oxidation in the absence of PS I is about half of that in the presence of PS I, suggesting that the rates of PS II oxidation via the oxidase and via PS I are rather similar. The oxidase, directly or indirectly, oxidizes the plastoquinone pool; after inhibition by KCN electron transfer from PS II to the plastoquinone pool can be fully restored by addition of quinones that are in redox contact with the pool. As the oxidase most likely is a component of the respiratory electron transport chain (see below), these results indicate the close connection between photosynthetic and respiratory electron flow in thylakoids of *Synechocystis*.

Another important feature is the observation that the fluorescence induction behavior of *Synechocystis* is very much dependent on the presence or absence of PS I. In wild-type *Synechocystis*, the amplitude of the fluorescence signal changes by about 50% upon reduction of Q_A. In the absence of PS I, the fluorescence yield is increased by a factor of three when Q_A is reduced. The low yield of variable fluorescence in wild-type vs. in the PS I-less mutant is due to a decrease in the absolute amount of variable fluorescence, rather than to a high F_o in the wild-type: as shown in Fig. 2, the amount of F_v is vastly different in wild-type (10 μg/ml chlorophyll) and the PS I-less mutant (2 μg/ml chlorophyll), at similar PS II concentration. This illustrates that nearby PS I is a powerful quencher of variable fluorescence. This phenomenon has an interesting parallel in thylakoids from higher plants (see [19]): in stacked thylakoids, where PS II and PS I are physically separated, a high variable fluorescence yield is found. Upon unstacking these thylakoids by removal of divalent cations at low ionic strength of monovalent cations [19], the variable fluorescence yield is decreased with little effect on F_o (for example, see [20]), and the lateral heterogeneity of PS II and

PS I disappears. The presence of PS I close to PS II thus is a significant drain on variable fluorescence yield. The efficiency of PS I in fluorescence quenching is fully compatible with the short fluorescence lifetime (and thus low fluorescence yield) of PS I components; in intact cyanobacteria [21] this lifetime is comparable to or shorter than in most photosynthetic eukaryotes (reviewed in [22]). These considerations explain the poor amplitude of variable fluorescence in wild-type cyanobacteria: in these organisms thylakoids are in permanently unstacked state, and PS I and PS II thus are assumed to be present in the same thylakoid domain.

In the absence of PS I, only component(s) which are sensitive to KCN appear to oxidize the PS II-reducible PQ pool. Even though the simplest explanation would be a single oxidase that directly or indirectly oxidizes the PQ pool, the presence of multiple oxidizing components cannot be excluded at this time, as long as they have similar KCN sensitivity. An important question is which oxidase(s) may oxidize the PS II-reduced PQ pool in *Synechocystis*. Candidates include a cytochrome aa_3-type cytochrome c oxidase (the terminal oxidase in respiration), as well as a quinol oxidase. In other prokaryotes, cytochrome b_o-type (*Escherichia coli*) and cytochrome aa_3-type (*Bacillus subtilis*) quinol oxidases have been found that structurally resemble the cytochrome c oxidase (see [23] for a review) and that also would be good candidates. In *Synechocystis* 6803, a gene cluster has been identified that resembles a *cox* operon (coding for cytochrome oxidase components) (G. Schmetterer, personal communication), and that also has similarity to the quinol oxidases from *E. coli* and *B. subtilis* (not shown). Deletion of part of this cluster in wild-type *Synechocystis* does not lead to a loss of the capacity to grow under photoautotrophic or photoheterotrophic conditions; also, upon deletion of the *cox*-like cluster the dark respiration rate remains significant (G. Schmetterer, personal communication). Even though at this moment we cannot say whether the oxidase directly oxidizes plastoquinol (as would be expected for a quinol oxidase), or acts via the cytochrome b_6f complex and cyt c_{553} (as would be expected for the cytochrome c oxidase), or whether both pathways are involved, it is clear that the oxidase is just about as effective an electron acceptor from PS II as the PS I complex is.

The results obtained indicate that the application of PS I-less cyanobacterial strains is valuable also to investigate interactions between photosynthetic and respiratory chains, and the development of a simple yet sensitive fluorescence method utilizing the redox state of Q_A as a probe may prove to be important for a further study of this interaction.

References

[1] Scherer, S. (1990) Trends Biochem. Sci. 15, 458–462.

[2] Peschek, G.A. (1987) in: The Cyanobacteria (Fay, P. and Van Baalen, C. eds.) pp. 119–161, Elsevier, Amsterdam.

[3] Scherer, S., Almon, H. and Böger, P. (1988) Photosynth. Res. 15, 95–114.

[4] Shen, G., Boussiba, S. and Vermaas, W.F.J. (1993) Plant Cell 5 (in press).

[5] Duysens, L.N.M. and Sweers, H.E. (1963) in: Studies on Microalgae and Photosynthetic Bacteria (Ashida, J. ed.) pp. 353–372, University of Tokyo Press, Tokyo.

[6] Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M. and Stanier, R.T. (1979) J. Gen. Microbiol. 111, 1–61.

[7] van der Bolt, F. and Vermaas, W. (1992) Biochim. Biophys. Acta 1098, 247–254.

[8] Vermaas, W.F.J., Ikeuchi, M. and Inoue, Y. (1988) Photosynth. Res. 17, 97–113.

[9] Vermaas, W., Vass, I., Eggers, B. and Styring, S. (1994) Biochim. Biophys. Acta (in press).

[10] Papageorgiou, G. (1976) in: Bioenergetics of Photosynthesis (Govindjee ed.) pp. 319–371, Academic Press, New York.

[11] Bryant, D.A. (1991) in: The Photosynthetic Apparatus: Molecular Biology and Operation (Bogorad, L. and Vasil, I.K. eds.) pp. 257–300, Academic Press, San Diego.

[12] Cao, J., Vermaas, W.F.J. and Govindjee (1991) Biochim. Biophys. Acta 1059, 171–180.

[13] Bennoun, P. (1970) Biochim. Biophys. Acta 216, 357–363.

[14] Robinson, H.H. and Crofts, A.R. (1983) FEBS Lett. 153, 221–226.

[15] Koulougliotis, D., Kostopoulos, T., Petrouleas, V. and Diner, B.A. (1993) Biochim. Biophys. Acta 1141, 275–282.

[16] Ogawa, T. (1992) Plant Physiol. 99, 1604–1608.

[17] Schluchter, W.M., Zhao, J. and Bryant, D.A. (1993) J. Bact. 175, 3343–3352.

[18] Fujita, Y. and Murakami, A. (1987) Plant Cell Physiol. 28, 1547–1553.

[19] Barber, J. (1980) FEBS Lett. 118, 1–10.

[20] Murata, N. (1969) Biochim. Biophys. Acta 189, 171–181.

[21] Bittersmann, E. and Vermaas, W. (1991) Biochim. Biophys. Acta 1098, 105–116.

[22] Holzwarth, A.R. (1991) in: The Chlorophylls (Scheer, H. ed.) pp. 1125–1151, CRC Press, Boca Raton, FL.

[23] Haltia, T. and Wikström, M. (1992) in: Molecular Mechanisms in Bioenergetics (Ernster, L. ed.) pp. 217–239, Elsevier, Amsterdam.