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Thermoluminescence and flash-oxygen characterization of the IC2 deletion mutant of *Synechocystis* sp. PCC 6803 lacking the Photosystem II 33 kDa protein

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The *psbO* gene product of Photosystem II (PS II), the so-called 33 kDa extrinsic protein, is believed to be closely associated with the catalytic Mn cluster responsible for light-induced water oxidation. However, this protein is not absolutely required for water-splitting and its precise role remains to be clarified. We have used flash-induced thermoluminescence and oxygen evolution measurements to characterize the process of water oxidation in the IC2 mutant of *Synechocystis* sp. PCC 6803 from which the *psbO* gene had been deleted by Mayes et al. (Mayes, S.R., Cook, K.M., Self, S.J., Zhang, Z. and Barber, J. (1991) Biochim. Biophys. Acta 1060, 1–12). The thermoluminescence results show that the extent of charge stabilization in the $S_2Q_A^-$ and $S_2Q_B^-$ states is reduced in the IC2 mutant to about 25–30% of that observed in the wild-type, suggesting that functional oxygen evolution occurs in a proportion of the *psbO*-less mutant cells. The stability of the $S_2Q_A^-$, but not that of the $S_2Q_B^-$, charge pair is markedly increased in the mutant. This points to a structural change of the PS II reaction center complex in the absence of the *psbO* gene product which affects the redox properties of the Q_A and Q_B acceptors to a different extent. The flash-induced oscillation of the B thermoluminescence band, arising from the $S_2Q_B^-$ and $S_3Q_B^-$ charge recombinations, is largely dampened in the mutant. This indicates that the ability of the water-oxidizing complex to reach its higher oxidation states, S_3 and S_4, is limited when the *psbO* gene product is absent. In agreement with the thermoluminescence results, flash-induced oxygen evolution shows a decreased yield and largely dampened oscillation pattern in the mutant. These results indicate that although the *psbO* gene product is not an absolute requirement for water oxidation its absence disturbs the redox cycling of the water-oxidizing complex and retards the formation of its higher S states. The rapid loss of thermoluminescence intensity during strong illumination of the mutated organism confirms its high susceptibility to photoinhibition. This effect is most likely the consequence of the limited rate of electron donation from the *psbO*-less water-oxidizing complex to the PS II reaction centre where the accumulation of highly oxidizing species may damage their pigment and protein surroundings.

Introduction

Photosystem II (PS II) is a multicomponent protein complex embedded in the thylakoid membrane of oxygenic photosynthetic organisms. It catalyses light-driven electron transport from water to plastoquinone and releases molecular oxygen as a side product (for recent reviews see Refs. 1–3). The reaction centre of PS II is composed of the D1 and D2 proteins which form a heterodimer with a close analogy to the reaction centre of purple bacteria [4].

The D1/D2 heterodimer binds the primary electron donor P_{680}, the primary electron acceptor pheophytin, the plastoquinone molecules which act as the secondary electron acceptors Q_A and Q_B. The D1/D2 complex also contains the redox-active tyrosine residues Tyr-Z and Tyr-D [1–3].

It is well established that a tetranuclear manganese cluster plays a central catalytic role in oxidizing water to molecular oxygen and protons by cycling through a series of oxidation states S_0 to S_4 [5,6]. The catalytic site of water oxidation is most probably directly associ-

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Abbreviations: Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1'-dimethylurea; PS II, Photosystem II.

ated with the PS II centre, possibly more with the D1 than with the D2 protein [7–9]. However, isolated D1/D2 reaction center complexes do not retain bound Mn and do not evolve oxygen [10], indicating that further protein components might be needed to form the catalytic Mn site.

In higher plants and eukaryotic algae, three extrinsic polypeptides are situated on the lumenal side of PS II, with apparent molecular masses of 33 kDa (*psbO* gene product), 23 kDa (*psbP* gene product) and 17 kDa (*psbQ* gene product) which have been implicated in oxygen evolution [11]. Various lines of experimental evidence, including their absence in cyanobacteria, has assigned non-catalytic roles to the 23 kDa and 17 kDa polypeptides [11–14]. These polypeptides seem to be involved in regulating Ca^{2+} and Cl^- levels in the vicinity of the Mn cluster [11,14].

In contrast, the 33 kDa protein which is also called MSP, the 'manganese stabilizing protein', is present in all oxygenic photosynthetic organisms including cyanobacteria. From in vitro studies it was concluded that the 33 kDa protein is not directly involved in Mn binding, since bound Mn and appreciable rates of oxygen evolution can be maintained in the presence of high, non-physiological Ca^{2+} and Cl^- levels, after washing the 33 kDa protein from PS II membranes [15,16]. On the other hand, under these conditions the characteristic period-four oscillation of flash-induced oxygen evolution is largely distorted, indicating a disturbed redox cycling of the Mn cluster [17]. Two of the four active-site Mn atoms are also quickly lost in the absence of the 33 kDa protein at physiological Ca^{2+} and Cl^- levels [15,18], leading to the idea that the 33 kDa protein is needed to stabilize the proper conformation of the catalytic site of water oxidation.

In vivo studies on *Synechocystis* sp. PCC 6803 [19] and *Chlamydomonas reinhardtii* [20] mutants lacking functional *psbO* genes also pointed to the necessity of the 33 kDa protein for oxygen evolution in vivo. However, recently Burnap and Sherman [21], Philbrick et al. [22] and also Mayes et al. [23] have constructed *Synechocystis* sp. PCC 6803 mutants in which the *psbO* gene had been deleted. Surprisingly, these mutants were able to grow photoautotrophically and evolved oxygen, albeit at a reduced rate [21,22], and were highly susceptible to photoinhibition [23]. Based on these results it was concluded that the 33 kDa protein is not essential for oxygen evolution in vivo [21–23] at least in this organism.

In order to obtain more precise knowledge regarding the function of the 33 kDa protein in vivo, we performed thermoluminescence and flash oxygen studies on the *psbO* gene-less IC2 mutant of *Synechocystis* sp. PCC 6803 constructed by Mayes et al. [23]. The results indicate that functional Mn cluster is present in only about one-third of the mutant PS II centres and even in those centres the ability to form the higher S states is retarded. We also confirm the increased susceptibility to photoinhibition of the mutant cells indicating a preferential damage to the donor side of PS II.

Materials and Methods

Strains and culture conditions. The *Synechocystis* sp. PCC 6803 strain used in our experiments was the glucose-utilizing strain of *Synechocystis* 6803-G [24] which is referred to throughout as *Synechocystis* 6803. It was routinely grown in BG-11 medium supplemented with 5 mM glucose at 28–31°C. Plate medium was supplemented as described by Pakrasi et al. [25]. Liquid cultures were grown in sterile conical flasks by being gently bubbled with air containing 5% CO_2. The IC2 mutant, lacking the *psbO* gene, was routinely grown in BG-11 medium supplemented with 5 mM glucose, in the presence of 25 μg ml^{-1} kanamycin to maintain the PS II lesion.

Photoinhibitory treatment. Wild-type and IC2 mutant cells in the exponential growth-phase ($A_{730\,nm}$ of 0.6–0.8) were harvested by centrifugation for 10 min at 4000 × *g* at room temperature. The chlorophyll *a* content was determined by methanolic extraction using the extinction coefficients as in Ref. 26 and the cells were diluted by fresh BG-11 medium to 50 μg Chl *a* ml^{-1}. Cells were subjected to various periods of illumination with white light, having an intensity of approx. 8000 μE m^{-2} s^{-1}, in a flat Petri dish during continuous stirring at room temperature.

Thermoluminescence measurements. Thermoluminescence was measured with a home-built apparatus as previously described [27] at 50 μg Chl *a* ml^{-1}. Before measurements cells were preilluminated with white light of 10 W/m^2 intensity for 30 s, followed by a 5 min dark-adaptation period at room temperature. Thermoluminescence was excited at 5°C by a series of single turnover flashes (3 μs) provided by a General Radio Stroboslave 1539-A Xenon flash at 1 Hz frequency. Following flash excitation samples were quickly cooled down in the dark to −40°C, after which the slow heating was initiated with a rate of 20 C° min^{-1} and thermoluminescence was detected. The electron transport inhibitor DCMU was added, when indicated, after 30 s preillumination in continuous light at the beginning of the 5 min dark adaptation period.

Steady-state oxygen evolution measurements. Steady-state rates of oxygen evolution were measured at 31°C using a Hansatech DW2 O_2 electrode at a light intensity of 3000 μE m^{-2} s^{-1}. Electron transport was supported either by 10 mM $NaHCO_3$ or by the artificial electron acceptor system of 1 mM 2,5-dimethyl-*p*-benzoquinone plus 1 mM potassium ferricyanide.

Flash oxygen measurements. Flash dependence of oxygen evolution was measured with an unmodulated

bare-platinum oxygen electrode as described earlier [28]. Cell suspensions were used at 50 μg Chl *a* ml^{-1} without artificial electron acceptors, and were preilluminated with a train of 50 flashes followed by 5 min dark adaptation. Oxygen evolution was induced by a series of short (3 μs) flashes provided by a General Radio Stroboslave 1539-A Xenon flash at 1 Hz frequency. Signals were detected by a home-built amplifier and a multichannel analyzer (ICA KFKI, 2.5–10 ms/point). The measurements were controlled by a Commodore 64 microcomputer.

Results

Thermoluminescence characteristics

Radiative recombination of positive charges stored in the S_2 and S_3 oxidation states of the water oxidizing complex with electrons stabilized on the reduced Q_A and Q_B acceptors of PS II results in characteristic thermoluminescence emission (for recent reviews, see Refs. 29, 30). The thermoluminescence intensity is proportional to the amount of recombining charges, whereas the peak temperature is indicative of the energetic stabilization of the separated charge pair: the higher the peak temperature the greater the stabilization [27]. With dark-adapted thylakoids or with PS II enriched membranes, illumination with one flash results in a single thermoluminescence band at around 30–40°C, called the B-band, which arises from the $S_2Q_B^-$ recombination [31,32]. If the electron transfer between Q_A and Q_B is blocked, e.g., by DCMU, the B band is replaced by the so called Q-band arising from the $S_2Q_A^-$ recombination at around 5–15°C [31,33].

In intact cyanobacteria the situation is more complicated and illumination with one flash leads to the appearance of two overlapping thermoluminescence bands from cells given only a short dark-adaptation period (not shown). This phenomenon might be related to the high reduction level of the plastoquinone pool in the dark which keeps a partially reduced population of both Q_A and Q_B. To obtain reproducible results, and homogeneous charge stabilization and recombination, cyanobacterial cells were illuminated for 30 s with white light followed by 5 min dark adaptation before flash excitation. After this pretreatment one-flash illumination consistently resulted in a single B thermoluminescence band which peaked at around 40°C in the wild-type cells while the Q-band, measured in the presence of DCMU, appeared at around 12°C (Fig. 1A). Single-flash induced thermoluminescence was also observed with the 33 kDa protein-less mutant. However, the intensity of the thermoluminescence from the mutant was only about 25–30% of that observed in the wild-type cells having the same chlorophyll concentration (Fig. 1B). In addition, the peak temperature of the thermoluminescence bands was also affected by the absence of the *psbO* gene product: the B-band appeared at around 35°C (Fig. 1B) somewhat lower than in the wild-type cells (Fig. 1A). In contrast, the Q-band appeared at 22°C in the mutant (Fig. 1B), i.e., 10°C higher than in the wild-type (Fig. 1A).

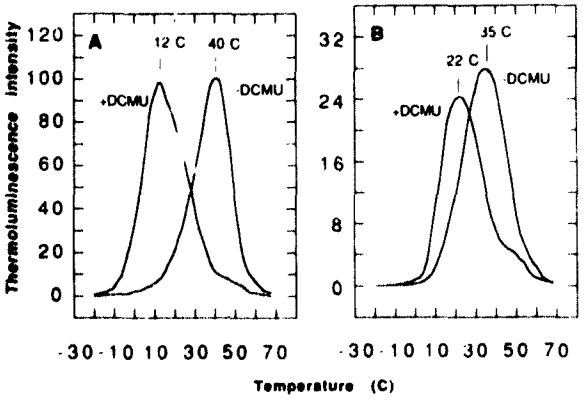

Fig. 1. Effect of *psbO* gene deletion on the single-flash induced thermoluminescence bands of *Synechocystis* 6803. Thermoluminescence of wild-type (A) and IC2 mutant of *Synechocystis* 6803 (B) was measured at 50 μg Chl *a* ml^{-1} after excitation with one flash in the absence or presence of 50 μM DCMU as described in Materials and Methods.

After illumination with two or more flashes both the $S_2Q_B^-$ and the $S_3Q_B^-$ recombinations contribute to the B thermoluminescence band [29,30]. The characteristic period-four oscillation in the intensity of the B thermoluminescence band, observed after illumination with a series of single turnover flashes, reflects the redox cycling of the S states [29,30]. The oscillation of the B-band intensity in the wild-type cells showed maxima after 2 and 6 flashes (Fig. 2A) which is the usual observation for PS II when a short dark adaptation is given prior to flash excitation. In contrast, in the mutant cells the B-band oscillation was much less pronounced and showed the first maximum after the third flash instead of the second flash (Fig. 2A). When the thermoluminescence intensities obtained after the first flash were normalized to the same value in the wild-type and mutant cells it became clear that thermoluminescence intensities after the second and higher number of flashes were primarily dampened in the mutant. A simulation of the B-band oscillation was also performed based on the model of Ref. 32 with the inclusion of the about 50% thermoluminescence yield of the $S_2Q_B^-$ recombination relative to that of the $S_3Q_B^-$ recombination [34]. This simulation shows that an increased miss probability at each S state transition or specifically at the $S_0 \rightarrow S_1$, or $S_1 \rightarrow S_2$ transitions would give a modification of the B-band oscillation (Fig. 2B) which is largely different from the experimental results (Fig. 2A). The modified oscillation pattern in the mutant cells can be best explained by the increased miss

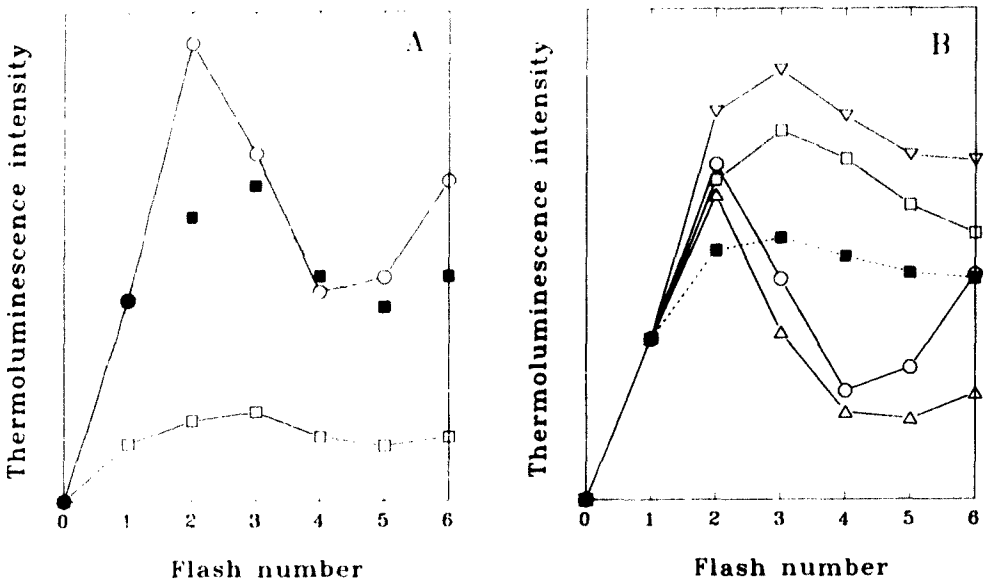

Fig. 2. Effect of *psbO* gene deletion on the flash-induced oscillation of the B thermoluminescence band of *Synechocystis* 6803. A: Experimental results: thermoluminescence of wild-type (●) and IC2 mutant of *Synechocystis* 6803 (□) was measured after excitation with a various number of flashes at 5 C. The oscillation of the B-band in the IC2 mutant cells is also shown after normalization of the 1st-flash intensity to the same value as obtained in the wild-type cells (■). B: Simulations: the oscillation of the B-band was simulated by assuming $S_0:S_1:S_2:S_3 = 0.25:0.75:0:0$; $Q_R:Q_B = 0.5:0.5$ initial distribution of states. For the oscillation of the wild-type cells 17% miss and 3% double-hit parameters were used (○). For the oscillation of the IC2 mutant cells either a 77% miss was assumed at the $S_0 \rightarrow S_1$ (△), $S_1 \rightarrow S_2$ (▽) transitions or 67% miss at the $S_2 \rightarrow S_3$ transition plus 47% miss at $S_3 \rightarrow S_0$ transition (■) or 37% miss at all transitions (□). Each simulated oscillatory pattern is shown after normalization for the intensity obtained after the first flash.

probability for the higher S state transitions, $S_2 \rightarrow S_3$ and $S_3 \rightarrow S_0$ (Fig. 2B).

Oxygen evolution

To obtain more direct information concerning S state turnovers in the absence of the 33 kDa protein flash-induced oxygen evolution was measured. The oxygen evolution pattern of cyanobacterial cells has been reported to show an unusual feature in comparison to higher plant chloroplasts: there is an electrochemical signal but no oxygen yield on the first flash [35,36] which was also observed both in the wild-type and mutant cells (Fig. 3). The flash pattern of oxygen evolution from the mutant showed a large degree of

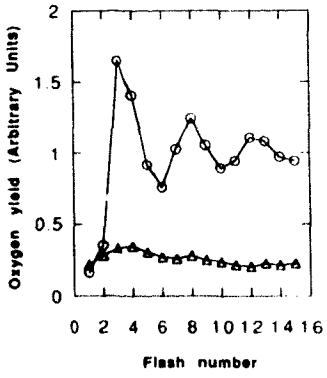

Fig. 3. Effect of *psbO* gene deletion on the flash-induced oscillation of oxygen evolution in *Synechocystis* 6803. Oxygen yield in a sequence of flashes was measured in the wild-type (●) and IC2 mutant cells (△) at 50 μg Chl *a* ml^{-1}. The frequency of the exciting flashes was 1 Hz.

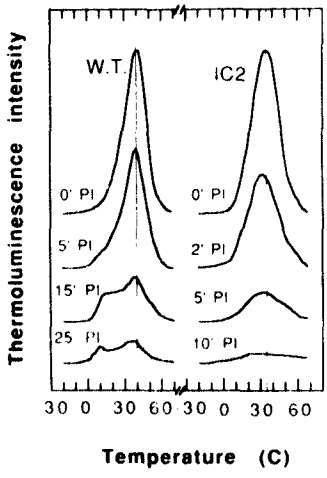

Fig. 4. Effect of photoinhibitory illumination on the B thermoluminescence band of the wild type and IC2 mutant cells. Wild-type and the IC2 *psbO*-less mutant cells were photoinhibited for various periods of time, as described in Materials and Methods, and thermoluminescence was measured after single-flash excitation as in Fig. 1.

dampening and a low steady state yield (Fig. 3) relative to that observed in the wild-type. This increased dampening of oxygen evolution in the IC2 mutant indicates a limited ability of S-state turnovers in agreement with the thermoluminescence results. Since oxygen is released during the final, $S_3 \rightarrow S_0$ transition, the occurrence of an increased miss at a particular transition can not be distinguished by model calculations from increased misses at each transition (not shown).

The steady-state rate of oxygen evolution, measured with a Clark-type electrode, was also decreased in the mutant cells. In the presence of CO_2 as a terminal acceptor, the oxygen-evolution rate in the mutant was 60–70% of that found in the wild-type. In contrast, when the artificial electron acceptor system of 2,5-dimethyl-*p*-benzoquinone plus ferricyanide was used the rate of oxygen evolution in the mutant reached only about 30% of that observed in the wild-type cells (not shown). These results agree well with previous findings [21–23].

Effects of strong illumination on PS II activity

Deletion of the *psbO* gene has recently been shown to increase the susceptibility to light-induced damage of PS II electron transport [23]. We applied thermoluminescence measurements to get a better insight into the mechanism of this phenomenon. As Fig. 4 shows, strong preillumination resulted in the gradual decrease of the B thermoluminescence band both in the wild-type and the IC2 mutant cells. However, with the mutant the loss of thermoluminescence intensity occurred much earlier than with the wild-type cells. Beside the intensity loss, photoinhibition also affected the shape of the single-flash induced thermoluminescence

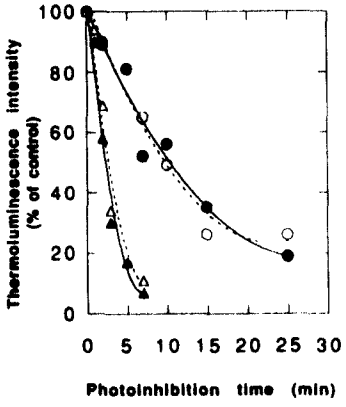

Fig. 5. Time-course of the decrease of thermoluminescence intensity during photoinhibition of wild-type and IC2 mutant cells. Thermoluminescence of photoinhibited cyanobacterial cells was measured as in Fig. 4 in the absence or presence of 50 μM DCMU. The intensity of the B (○, △) and Q thermoluminescence bands (●, ▲) is plotted as a function of the time of photoinhibitory treatment for wild-type (○, ●) and IC2 mutant cells (△, ▲).

curves. In the wild-type cells the peak position of the B-band was hardly changed, but after 10–15 min of photoinhibitory treatment a peak appeared approximately at the position of the Q-band (Fig. 4), indicating a partial interruption of the Q_A to Q_B electron transfer. In the IC2 mutant cells the peak position of the B-band was slightly shifted to lower temperatures during photoinhibition but, unlike the wild-type, the presence of two distinct thermoluminescence components was not obvious (Fig. 4). The intensity of the Q thermoluminescence band, measured in the presence of DCMU, was decreased by the strong illumination to a similar extent as that of the B-band both in the wild-type and the IC2 mutant (Fig. 5). Fig. 5 also shows that with the IC2 mutant thermoluminescence was lost about 3-times faster than in the wild-type as a result of treatment with photoinhibitory light.

Discussion

In this paper we describe thermoluminescence and oxygen evolution studies performed on the IC2 mutant of *Synechocystis* 6803 which lacks the *psbO* gene product (the 33 kDa extrinsic protein). The IC2 mutant, created by Mayes et al. [23], is able to grow photoautotrophically, as has been shown for other *psbO*-less *Synechocystis* mutants [21,22]. This indicates that the 33 kDa protein is not absolutely required for water-splitting in *Synechocystis* 6803. The mutant cells, however, possess a decreased PS II activity as revealed by variable fluorescence and steady-state oxygen evolution measurements [21–23]. This characteristic might arise from the presence of fewer PS II centres with an active water-oxidizing complex and/or from a slower rate of electron transfer in the individual reaction centres which lack the 33 kDa protein.

Our results show that thermoluminescence intensities from the $S_2Q_B^-$ and $S_2Q_A^-$ charge recombinations are only 25–30% in the IC2 mutant compared with that observed in the wild-type cells having the same chlorophyll *a* concentration (Fig. 1A, B). Moreover, the steady-state thermoluminescence intensity also decreased to a similar extent in the mutant (Fig. 2A) as compared with the wild-type cells. A decrease in electron-transfer rate or increase in misses in each PS II complex of the mutant cells could explain the increased dampening of the thermoluminescence oscillatory pattern, but not the loss of steady-state thermoluminescence intensity. This latter observation indicates that charge accumulation occurs in fewer water-oxidizing complexes. The alternative possibility that the stable reduction of the Q_A or Q_B quinone acceptors is prevented in the absence of the *psbO* gene product, is unlikely. Indeed, in vitro experiments have shown that the removal of the 33 kDa protein causes the release of the four catalytic Mn atoms, under physiological

ionic conditions, concomitant with the loss of thermoluminescence intensity [3]. Extrapolation from these results indicates that about 70–75% of the reaction centres do not retain fully active Mn cluster in the IC2 mutant. This conclusion is supported by the reduced rate of steady-state oxygen evolution which is only about 30% in the mutant relative to the wild-type when PS II activity is assayed in the presence of artificial acceptors. The fluorescence measurements of Philbrick et al. [22] have also indicated that at least 40% of the PS II centres are inactive in oxygen evolution in the absence of the 33 kDa protein.

The flash-induced oscillation of thermoluminescence shows a largely dampened pattern in the mutant cells which affects mainly the B-band intensities measured after two and higher number of flashes. This indicates that there is a reduction in the effectiveness of higher S state transitions ($S_2 \rightarrow S_3$ and $S_3 \rightarrow S_0$). A similar situation has been observed when the 33 kDa protein was depleted in vitro in the presence of high Cl^- concentrations [17,18]. However, with physiological Cl^- (and Ca^{2+}) levels the absence of the 33 kDa protein almost completely blocks the S_3 to S_0 transition in vitro [18,37]. This difference between the in vivo and in vitro systems might indicate that there is either an additional damage to the water-oxidizing complex by salt washing used to remove the 33 kDa protein or unusually high Cl^- and Ca^{2+} levels in the intact *Synechocystis* 6803 cells. Relevant to the latter possibility is the observation by Philbrick et al. [22] that under conditions of Ca^{2+} depletion where wild-type growth is unaffected the *psbO*-less mutant of *Synechocystis* 6803 was unable to grow at all.

The above findings point to the conclusion that in the absence of the *psbO* gene product the water-oxidizing complex is inactive in a population of PS II centres in *Synechocystis* 6803. In the remaining population of centres water oxidation occurs but the formation of the higher S states is retarded.

Deletion of the *psbO* gene also induced a shift of the Q thermoluminescence band to a higher temperature. Such a shift of the Q-band is characteristic of an increase in the stability of the $S_2Q_A^-$ charge pair. In contrast, the peak position of the B-band is almost the same in the wild-type and mutant cells, indicating no major change in the energetic stability of the $S_2Q_B^-$ charge pair. Since the two recombining charge pairs have the same donor (S_2) and different acceptor (Q_A or Q_B) components, it can be concluded that the absence of the *psbO* gene product exerts an effect on the acceptor side of PS II. A very similar stabilization of the $S_2Q_A^-$, but not the $S_2Q_B^-$, recombination has also been observed upon removal of the 33 kDa extrinsic protein using in vitro systems [38,39]. This effect, which can be reversed by rebinding the isolated 33 kDa protein, was interpreted as showing a reversible structural change of the PS II reaction centre complex which affects the redox properties of the Q_A and Q_B acceptors to a different extent [38,39]. At first sight, it seems surprising that the absence of an extrinsic protein which binds to the lumenal side of the reaction centre could affect redox components located close to the stroma side of the reaction centre. However, there is a good evidence to suggest that the 33 kDa protein is closely associated with the D1 and D2 reaction-centre proteins [8]. Moreover, there is already a precedent in the literature for an acceptor side-effect induced by the absence of the 33 kDa protein, namely the altered affinity of various artificial electron acceptors to the Q_B site after $CaCl_2$ wash [40]. In addition, 77 K fluorescence characteristics of the *psbO*-less *Synechocystis* 6803 mutant reported by Burnap and Sherman [21] indicate an altered conformation of CP47 which is a membrane-spanning protein in close association with the reaction centre complex. Overall, our findings confirm the previous results obtained from in vitro experiments [38,39] and point to the structural function of the 33 kDa protein in keeping the optimal conformation of the PS II reaction centre.

In agreement with the thermoluminescence results, flash-induced oxygen evolution exhibits a low yield and largely dampened oscillatory pattern in the IC2 mutant cells (Fig. 3). This is consistent with a decrease in the number of PS II centres having an active water-oxidizing complex and with the retarded S-state turnovers in the partially active centres.

Based on steady-state oxygen evolution measurements, it was concluded that the IC2 mutant exhibits an increased susceptibility to photoinhibition [23]. Our thermoluminescence results confirm this finding and show that the loss of charge stabilization in the $S_2Q_A^-$ or $S_2Q_B^-$ states, due to photoinhibitory illumination, occurs about 3-times faster in the IC2 mutant than in the wild-type cells. Photoinhibition of wild-type *Synechocystis* 6803 not only decreased the intensity of the B-band but also induced the accumulation of a component at the position of the Q-band.This agrees with previous thermoluminescence studies performed with intact *Chlamydomonas* cells [41] and most likely indicates an inhibition of the Q_A to Q_B electron transfer by the strong illumination. In the IC2 mutant, the induction of the Q-band was not obvious during photoinhibition, although a small downshift was observed in the peak position of the B-band.

Lesions of donor-side electron transport in PS II are well known to enhance the susceptibility to photoinhibition [42,43]. In the absence of efficient electron donation from the water-oxidizing complex highly oxidizing species, $Tyr\text{-}Z^+$ or $P680^+$, are accumulated in the reaction centre [44] and can damage their protein and pigment surroundings leading to the degradation of the D1 protein [45,46]. Thus, the accelerated photo-

inhibition of the IC2 mutant cells is most likely related to the perturbation of the function of the water-oxidizing complex in the absence of the *psbO* gene product.

Acknowledgements

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